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Studies to evaluate methodologies used for determining heavy metal content in polyethylene terephthalate food packaging

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The student author and the program of study committee are solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

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ABSTRACT

Increased consumer awareness of contamination in food-contact packaging has raised global concerns due to the potential of environmental contamination from packaging upon disposal after the service lifetime. Contamination in virgin and recycled polymers used for food-contact packaging has necessitated the development of analytical methods that identify and quantify heavy metals. Heavy metal contaminants in food-contact plastics have the potential to cause health issues if leaching were to occur. Sample preparation and analytical methods were evaluated to quantify heavy metal content in polyethylene terephthalate (PET). Since PET is one of the most widely used polymers for food-contact applications, accurate quantification of heavy metal content is essential to ensuring consumer safety. The two published acid digestion methods yielded incomplete sample digestion of PET, thus, additional methods were required for proper PET analysis. To circumvent this, modified microwave-assisted acid digestion methods were developed, which result in complete PET digestion and produce visually clear solutions. Analysis of the complete PET digests by inductively coupled plasma-optical emission spectrometry (ICP-OES) resulted in lead and antimony content values statistically higher than the two previously mentioned methods. To evaluate the ability of non-destructive methods to quantify heavy metal content in PET food packaging, X-ray fluorescence (XRF) was compared with ICP. Traditional analytical methods such as ICP are time-consuming and expensive processes. Moreover, testing if XRF technology can provide a means for monitoring heavy metal content in thin plastics would greatly reduce the frequency of sample testing by traditional methodology. Results from this analysis suggests that it is possible to evaluate thin plastic samples by developing a statistical model that estimates ICP data from XRF outputs. This research shows that XRF technologies

can be applied to online systems for real-time monitoring of heavy metal contamination in food packaging plastics. The results of these studies indicate that while food-packaging plastics should be regarded as safe, previously published research has underestimated the heavy metal contamination in polymers used for food packaging. This is of concern when considering end-of-life disposal for food packaging with regulatory threshold levels for specific and total heavy metal content.

CHAPTER 1: GENERAL INTRODUCTION

Introduction

The plastics industry plays an important role in the daily lives of consumers. Plastic food packaging offers a light, convenient way to transport and protect a variety of products. Companies are searching for ways to make packaging more cost effective and user friendly. This typically means more plastic and less glass or paper. Polyethylene terephthalate (PET) is the most widely used polymer for food contact packaging applications. The increased use of PET packaging has led to increased dumping in landfills [1]. Efforts to increase consumer recycling efforts have seen limited impact, as of 2015 only 30% of PET containers are recycled annually [2]. In addition to the environmental impacts are the health concerns associated with plastics packaging.

Plastics have the potential to leach heavy metals into the environment when improper disposal occurs [3]. Heavy metals in plastics may be introduced unintentionally by co-mingling with mixed waste streams or intentionally as catalytic compounds which are used to aid in the polymerization reaction [3]. Elements such as lead, cadmium, hexavalent chromium, antimony, and mercury are toxic when consumed in high doses and are associated with end-of-life concerns in public water sources if not disposed of properly [3]. As such, it is important to monitor heavy metal content in these materials to below regulatory thresholds to reduce risk to the environment and consumer.

Methods to evaluate heavy metal contaminants in packaging materials have greatly improved in recent years [4, 5]. Both sample preparation and analytical methods for the determination of heavy metals in plastics used to take several days to complete [6]. However, the use of modern sample preparation techniques such as microwave digestion reduces the time

from days to several hours. Limited research has been done on the effectiveness of standard methods for sample preparation or analytical methods to be used [7-9]. As such, there is potential for large variation between methods, even when analyzing the same material.

LITERATURE REVIEW

Plastics are a group of polymers with features that differentiate them from other polymeric materials such as rubbers, fibers, and adhesives [10]. The main difference between plastics and other polymeric materials is that plastics can be heated and made to flow, using controlled heat and pressure, and become solid in the final product after cooling [10]. Plastics offer a barrier to environmental factors such as moisture, light, and gas exchange [10]. In addition, plastics provide a lightweight, damage-resistant barrier to protect perishable goods such as fresh produce. Plastics commonly associated with the packaging industry include polyethylene terephthalate (PET), polystyrene (PS), polypropylene (PP), polyvinyl chloride (PVC), and polyethylene (PE).

Plastics fall into one of two groups, thermosets and thermoplastics. A thermoset polymer has high levels of cross-linking, irreversible covalent bonding, and cannot be re-heated and re-shaped once it is formed. Reheating a thermoset past its decomposition temperature causes degradation of the material either through cracking or charring [11]. A thermoplastic polymer can undergo repeated heating and reforming due to its relatively simple structure and lack of cross-linking bond configuration [10]. Examples of thermosets are fiber-reinforced composites and polymeric coatings [10]. Thermoplastics are the most commonly used group of plastics for food-contact applications. Water bottles and milk jugs, utilize two of the most widely used thermoplastic polymers, PET and PE, respectively.

PET is a semi-crystalline thermoplastic polymer that has been widely adopted due to its low cost, relatively low permeability to oxygen and carbon dioxide, high strength to weight ratio, and excellent clarity [12]. Polymerization of PET is achieved through one of two polycondensation reactions. The first process (Fig. 1) uses ethylene glycol (EG) and terephthalic acid (TPA), the oligomer of TPA and EG is catalyzed by Lewis acidic metals such as antimony, germanium, or titanium to form the PET homopolymer [13].

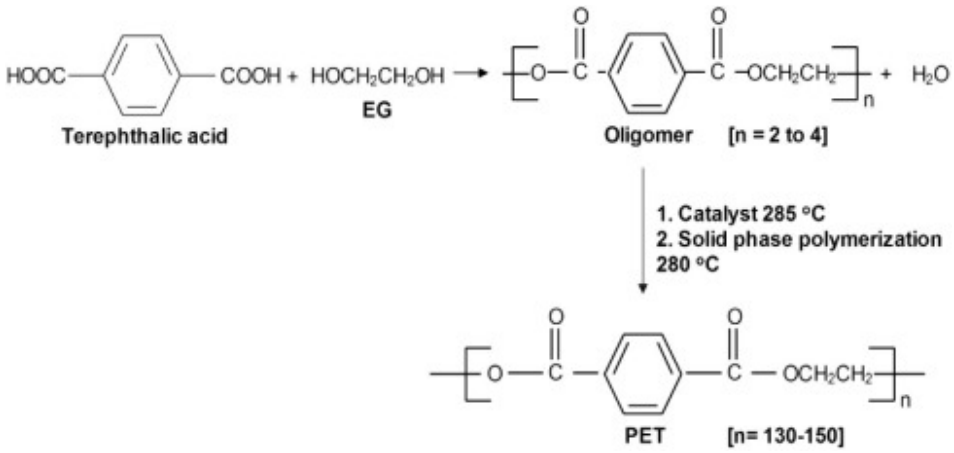

Figure 1. Synthesis and polymerization of PET homopolymer from TPA and EG [14]

The second polycondensation reaction (Fig. 2) for the formation of PET involves the use of TPA, EG, and 1,4 cyclohexane dimethanol (CHDM) co-monomer [15]. While the products of Figures 1 & 2 are both PET, the second reaction is considered a copolymer because it contains a second monomeric group (CHDM). PET processed with CHDM tends to be more amorphous, have a higher glass transition temperature (T_g), lower melt temperature (T_m), and is widely used for bottled beverages, such as water and soft drinks [15].

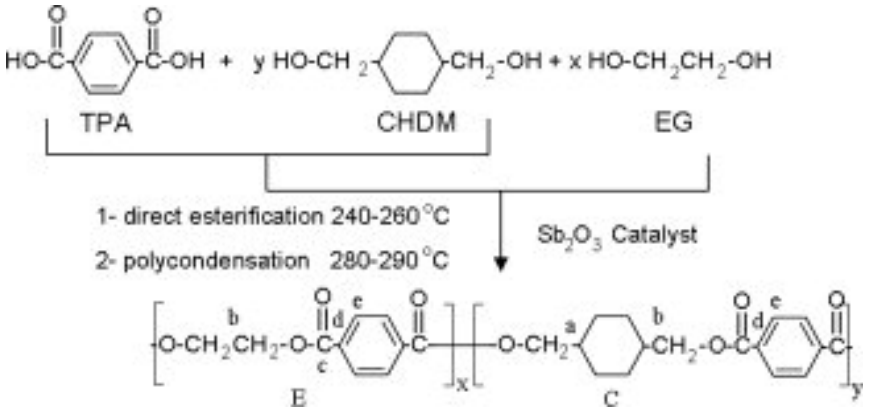

Figure 2. Synthesis and polymerization of PET copolymer from TPA, EG, and 1,4 cyclohexane dimethanol [15]

In 2015, of the 6.0 billion pounds of PET produced in the United States, 1.8 billion pounds were recycled (30% recycling rate) [2]. Post-consumer polyethylene terephthalate (i.e., recycled; RPET) can be re-introduced into the supply stream for use in beverage bottles, thermoformed food containers, as well as a variety of non-food contact applications such as fiber [2]. The cost savings, barrier properties, and recyclability of thermoplastics offer many benefits to both consumers and manufacturers. However, there are many unintended impacts that result from the misuse of these materials.

Environmental impact of heavy metals in plastics

Heavy metals are naturally occurring substances that are typically found at lower levels in the environment, but can build up over time from consumer and manufacturing sources such as fossil fuel combustion, oil refining, mining, and welding [16, 17]. The term 'heavy metal' is not well defined, but is commonly considered to be higher density (>5 g/cm^3) and higher atomic weight atoms that fall into the transition metal and metalloid categories on the periodic table of elements and are toxic [17]. Heavy metals are a cause for concern as their presence in recycled plastics typically points to contamination from unintended co-mingling sources such

as electronic waste, pigments or dyes, and products produced before regulations were implemented [3].

Environmental and health concerns surround the presence of specific heavy metals lead (Pb), cadmium (Cd), hexavalent chromium (Cr (VI)), and mercury (Hg) in all packaging materials. The heavy metals listed are considered carcinogens and have been associated with a number of other adverse health conditions [16, 18]. Antimony (Sb) is also of concern due to its toxicity level. Antimony is used as a polymerization catalyst in PET and residual concentrations of antimony in PET are typically between 150 – 300 parts-per million (ppm; mg/kg) [7]. Abuse studies showed that antimony has the capability to migrate into liquid substrates under extreme conditions that are not considered normal storage [7, 19]. Examples of these extreme conditions include storage in a hot car for several weeks, in direct sunlight for extended periods of time, or microwaving a bottle of water [7, 19]. While these abuse studies represent the most extreme cases for the migration of contaminants from plastics, there are also concerns with the degradation of plastics in landfills [20]. The length of time it takes for degradation of polymer material in a landfill depends on a variety of environmental factors such as light, heat, moisture, biological activity and chemical conditions [20]. It is possible that the slow degradation of these plastic bottles could release heavy metals such as lead, cadmium, hexavalent chromium, mercury, and antimony into ground waters. As such, it is important to monitor heavy metal content in plastics to ensure that environmental contamination does not occur.

Diversion of plastic waste decreases stress on both the environment as well as reducing the rate at which landfills become full. Safety concerns surrounding leaching of heavy metals from landfills and waste sites into the environment led to the of introduction of model

legislation, set forth by the Coalition of Northeastern Governors (CONEG), which seeks to eliminate or reduce the amount of heavy metals lead, cadmium, hexavalent chromium, and mercury in packaging materials. The heavy metal content requirements, as set forth by the CONEG in 1992, were adopted in Europe under the European Union's Packaging and Packaging Waste Directive (94/62/EC) in 1994, Waste Electrical & Electronics Equipment (WEEE) Directive in 2002, and the Restriction of Use of Certain Hazardous Substances in Electrical and Electronics Equipment (RoHS) Directive also in 2002 [21, 22].

The development of legislation like the CONEG regulations and the EU Packaging Directive was a response to growing concerns that toxic heavy metals had the potential to end up in packaging materials and leach into the environment at the end of life [23]. The focus on packaging materials was due to concerns regarding degradation in landfills and as artifacts in the environment when improperly disposed. Over time, the concern is that these toxic heavy metals will be released into the environment through various routes including air pollution from incineration and landfill leachates [23]. In addition, marine environments are also at risk for leaching of heavy metals resulting from marine litter in the oceans and waterways [24].

Toxicity of heavy metal exposure

Long-term exposure to lead has been determined to be a known carcinogen in humans [17]. High levels of lead exposure has been linked to serious health effects including chronic renal failure, miscarriages in women, and severe brain damage in children due to a weaker blood-brain barrier, when compared to the adult blood-brain barrier [17]. Due to the severity associated with high levels of lead intake, the EPA has set a limit of 15 parts-per-billion (ppb; μg/kg) in drinking water [16].

Exposure to cadmium has been shown to be carcinogenic when consumed at high dosage levels [16]. Health concerns such as severe lung damage in smokers, kidney disease, severe stomach irritation, and bone damage are all associated with cadmium exposure. Research suggests that lower levels of consumption over extended periods of time, such as through inhalation may be more detrimental than previously expected [17]. The EPA regulatory limit on cadmium concentrations in drinking water is 5 ppb [16].

Chromium exposure is not immediately a cause for concern, as chromium has two different oxidation states. Trivalent chromium (Cr (III)) is an essential nutrient, meaning that humans need to consume it in their diet due to the inability to synthesize [16]. Hexavalent chromium compounds are toxic and known human carcinogens [16]. Previous studies suggest that extended hexavalent chromium exposure is associated with developmental issues in children, liver damage, as well as reproductive problems in men [25]. The EPA regulatory limit on total chromium is 100 ppm in drinking water [16]. The limit is higher due to the two different oxidation states, relative abundance, and their respective toxicities.

Mercury combines with other elements to form organic and inorganic mercury compounds [16]. Compounds such as mercuric chloride and methylmercury are known human carcinogens. Mercury toxicity is associated with neurological and psychological symptoms as well as brain and kidney damage, birth defects, and cancer [16, 17]. The most common modes of human exposure are through food sources like seafood and dental amalgams in parts of the world where they are still used [17]. Mercury is heavily regulated due to its highly toxic nature. The EPA regulatory threshold in drinking water is 2 ppb and the Food and Drug Administration (FDA) regulates methylmercury in seafood to 1 ppm [16].

Antimony toxicity is associated with serious health issues such as lung disease from inhalation, severe abdominal pain, ulcers, and diarrhea from oral ingestion, as well as carcinogenic effects from prolonged exposure [18]. Occupational exposure with workers involved in the production of antimony trioxide, mining, smelting, as well as coal-fired facilities are considered to be the most common modes of exposure [18]. However, consumers can also be exposed via contaminated water, food, and soil contact [26]. While antimony content in packaging is not regulated not regulated in the United States, the European Union regulates antimony concentrations (EU 10/2011) to a maximum of 350 ppm [27, 28]. Due to these types of production, the level of toxicity, and the possibility of residual antimony ending up in public water sources, the EPA has set a regulatory threshold of 6 ppb in drinking water [29].

Heavy metal contamination in plastic packaging

Heavy metals are introduced into polymeric materials in a variety of ways by either intentional or unintentional mechanisms. Heavy metals may be added as catalysts, plasticizers, antimicrobial agents, or flame inhibitors [6]. The most prevalent intentional introduction is in the form of metal catalysts, which decrease the energy required to initiate and propagate the polymerization reaction necessary to form the polymer chain. In PET, heavy metal catalysts are introduced in the form of a metal oxide such as antimony trioxide (Sb_2O_3) and are often found in plastics as residual catalyst from the polymerization reaction [13]. Of the available catalysts, antimony trioxide is the most common catalyst used for PET due to its relatively low cost, lack of color introduction, and excellent catalytic activity [30]. Other heavy metal catalysts used for the polymerization of PET include germanium dioxide (GeO_2) and titanium dioxide (TiO_2) [31].

Quantification of heavy metals in plastics

To determine the concentration of heavy metals in a solid sample, methods that liberate the heavy metal contaminants from the material are commonly used. Quantification of elemental content in polymeric materials falls into one of two types of analysis: destructive and non-destructive. Non-destructive methods involve the direct analysis of the sample, without disturbing the integrity of the material. Destructive analysis is commonly referred to as digestion and is carried out by completely degrading the polymer structure and capturing the degraded material into a solution which can be analytically evaluated.

Digestion of polymers can be achieved by several methods, one of which is an open-vessel wet-ashing method, which involves multiple heating steps on a hotplate, in a mixture of concentrated acids. Reagents commonly used for wet-ashing methods include nitric acid (HNO_3), perchloric acid ($HClO_4$), sulfuric acid (H_2SO_4), and hydrogen peroxide (H_2O_2) [8, 32]. Wet-ashing techniques are performed over several hours, require constant attention, special hoods when perchloric acid is involved, and greater acid volumes [8, 32]. While industry labs more commonly use wet-ashing techniques, closed-vessel microwave-assisted acid digestion (MWD) methods are more commonly used in academic and research settings.

Digestion protocols, which utilize microwave technologies, are more rapid and have fewer opportunities for sample contamination, since they are carried out in closed vessels. Using acid digestion, it is possible to completely degrade the polymer matrix, obtaining a clear solution, which can be analyzed by several techniques including inductively coupled plasma (ICP) and atomic absorption spectroscopy (AAS). Polymer solutions, which are free of any

particulate material, are necessary to quantify heavy metal content using ICP or AAS as will be demonstrated below.

One of the most common analytical methods for the determination of heavy metal content is ICP. Depending on the concentration of the elements in the sample, an ICP optical emission spectrometer (ICP-OES) or ICP mass spectrometer (ICP-MS) is used. ICP-OES is commonly used for higher concentration (ppm) samples while ICP-MS is more commonly used when a much lower detection limit (ppb) is desired. ICP-MS has lower detection limits (i.e. higher sensitivity) than ICP-OES because the mass spectrometer measures the ions more directly than optical emission [33]. Optical emission spectrometers measure the light emitted by the ions [33]. Heavy metals in packaging materials typically are in the low-to-mid ppm range, thus ICP-OES is the more appropriate application as the sensitivity levels of ICP-MS are typically not necessary [34].

ICP-OES quantifies elemental concentrations through the detection of wavelength emissions. This is the result of sample introduction through the argon plasma, which causes the electrons of an atom to jump to a higher energy state [35]. Almost instantaneously (10^{-8} s) the electron falls back to a lower energy state [35]. The difference in energy levels of the two states is emitted as electromagnetic radiation in the form of a characteristic wavelength, this phenomena is known as excitation [35, 36]. ICP-OES detects wavelength emissions in the vacuum ultraviolet (VUV; 120-185 nm), ultraviolet (UV; 185-400 nm), visible (VIS; 400-700 nm), and near infrared (NIR; 700-850 nm) regions [36]. ICP software can quantify the elemental concentrations contained in a sample by evaluating counts at discrete energy levels (wavelengths) associated with each element [35]. This is done by referencing the wavelength

of an unknown sample emission to a series of known elemental standards [35]. However, the element of interest must be contained within the series of standards for quantification.

The ICP-OES instrumental components are comprised of a sample introduction system, the torch assembly used to sustain the argon plasma, and a spectrometer for the detection of wavelength emissions. The argon plasma acts as an atomization and excitation source in that all organic matter that enters the plasma is destroyed by the 8,000 Kelvin (K) ionized gas [36]. After atomization, all bonds are broken and the remaining atoms gain energy via collisions and emit wavelengths that can be separated and detected by the spectrometer [36].

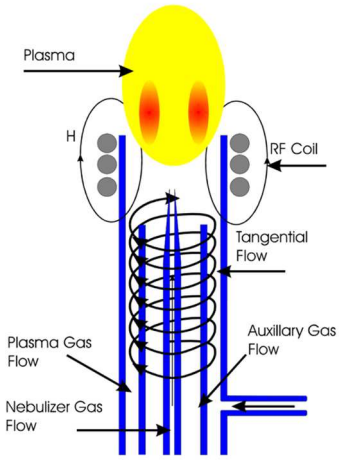

Figure 3. Schematic of a torch used for inductively coupled plasma [30].

The time required for heavy metal quantification using ICP-OES, ICP-MS, or AAS is three to four hours for a small number of samples or a day or more for larger sample sets due to the need for sample preparation, decomposition, and subsequent analysis [6]. A more rapid, but generally less sensitive, analytical method for the quantification of heavy metal content is X-ray fluorescence (XRF) spectroscopy and its variants.

XRF is a widely accepted analytical technique for determining elemental concentrations in a variety of sample matrices such as painted surfaces, coal, soil, and plastics [37-41]. When sufficient energy is applied to the sample by incident X-ray, an inner-orbital electron of an atom is ejected. An outer shell electron will fill the vacancy and emit an X-ray photon with an energy that is unique to each element [40]. Like ICP-OES, XRF is a comparative method meaning that standards of known concentration must be used to calibrate

the instrument and provide a reference for counts-per-second (or intensities) for unknown samples.

There are two types for XRF analyzers: wavelength dispersive XRF (WDXRF) and energy dispersive XRF (EDXRF). XRF exposes samples to a primary X-ray, which is provided by the instrument, and detects secondary or characteristic X-rays which are emitted by the elements exposed to the primary X-ray [42]. The WDXRF analyzer uses a diffraction crystal to separate X-rays according to wavelength [42]. The EDXRF analyzer directs secondary X-rays to a detector that converts the X-ray into a voltage signal [42]. WDXRF analyzers have a larger footprint, lower detection limits, but are more expensive than EDXRF analyzers. EDXRF has the ability to quantify specific or multiple elements simultaneously. Analysis by all XRF instrumentation is non-destructive, requires very little sample preparation, and takes between one and three minutes to complete. Like all instrumental methods, XRF has its drawbacks. For reliable quantification of elements by XRF, as sample must be of sufficient thickness. The common term for this property is “infinite thickness” [42].

The term infinite thickness describes the depth that the primary X-ray must travel into and out of the sample [42]. A sample analyzed by XRF should be thicker than the depth that the X-rays can travel into a material as samples that are of less than infinite thickness are partially transparent to incident X-ray and sufficient excitation may not occur [42]. The calculation for infinite thickness is determined, in part, using the Beer-Lambert Law (Beer’s Law; Eq. 1). The mass attenuation coefficient (MAC; Eq. 2) is obtained experimentally and are obtained from National Institute of Standards and Technology (NIST) Tables for each element [43]. In addition, the infinite thickness $\left(t_{inf}; \text{Eq. } 3\right)$ changes based on the density of a

given material [42]. Using equations 1-3, the infinite thickness for PET was determined (Eq. 4) to be 21 mm.

1. $I_f = I_0 e^{-\mu\rho d}$
2. $\mathrm{MAC} = \mu_{\mathrm{i,e}} * \mathrm{C_i} + \mu_{\mathrm{j,e}} * \mathrm{C_j} + \mu_{\mathrm{k,e}} * \mathrm{C_k}$
3. $\frac{1}{\mu\rho} = t_{inf}$
4. $\mu = 0.625_{\mu_{Sb-C}} + 0.042_{\mu_{Sb-H}} + 0.333_{\mu_{Sb-O}}$
   $(0.625)(0.28) + (0.042)(0.40) + (0.333)(0.47)$
   $= 0.348$
   $= \frac{1}{0.348 * 1.38} = 21 \text{ mm}$

PET: $C_{10}H_8O_4$
ρ= Density of PET: 1.38 g/cm^3
$\mu_{\mathrm{x,e}}$= Leroux Table MAC of PET in cm^2/g
- Sb through Carbon = 0.28
- Sb through Hydrogen = 0.40
- Sb through Oxygen = 0.47

C_x= Concentration of "*i-k*", as a percent
- C: 12*10 = 120 or (62.5%)
- H: 1*8 = 8 or (4.2%)
- O: 16*4 = 64 or (33.3%)

Plastics commonly used for food contact packaging are between 0.3 and 0.6 mm thick, thus, the sample thickness required to properly analyze PET by XRF is generally not obtained for food packaging applications, and a correction factor is likely required for accurate measurements. Analyzing samples that are thinner than infinite thickness results in a lower counts-per-second (cps) or weaker intensity than would be observed from a sample that is of sufficient thickness due to lack of depth for excitation by primary X-ray. A weaker recorded intensity corresponds to a concentration output, which is lower than the actual concentration.

The ability to accurately and precisely report heavy metal concentrations in food packaging plastics is essential to the safety of both consumers as well as the environment. With the wide variety of sample preparation and analytical methods available for heavy metals analysis, understanding the advantages and disadvantages of each is integral to accurate quantification of heavy metals. If inaccurate results are presented and higher than usual contamination is observed (e.g. greater than 100 ppm total CONEG-regulated heavy metals), the outcome could result in hazardous health and/or environmental conditions. In addition, if

future compliance levels are decreased from the current level of 100 ppm for lead, cadmium, hexavalent chromium, and mercury, the accuracy of heavy metals quantification and reporting becomes increasingly important.

Summary

Polyethylene terephthalate is one of the most widely used thermoplastic polymers due to its relatively low cost as well as good barrier, and optical properties. Heavy metals are introduced into polymer matrices either through intentional addition in the form of catalysts or through contamination sources. Due to heavy metal toxicity in humans, lead, cadmium, hexavalent chromium, and mercury are regulated to a maximum of 100 ppm in packaging materials. While antimony is not regulated in packaging in the United States, European Union regulations allow up to 350 ppm [27, 28]. Sample preparation methods commonly used for degrading the polymer matrix are conventional wet-oxidation or microwave digestion methods, in the presence of strong acids. Once digested, quantification of these elements is carried out via inductively coupled plasma based methods or by atomic absorption spectroscopy. A less sensitive but non-destructive method that can be used for the determination of heavy metals in plastics is X-ray fluorescence (XRF). Overall, the ability to accurately and precisely report heavy metal concentrations in food packaging plastics is essential to ensure the safety of both consumers as well as the environment. Results of these studies showed that choice of sample preparation method may have a greater impact on heavy metals quantification than previously expected. These studies also assessed the viability of XRF to analyze heavy metals in an online system. It was shown that a statistical estimation model can be used to develop a correction factor for XRF sample analysis when samples are below the infinite thickness required for accurate testing.

THESIS ORGANIZATION

The second chapter of this thesis comprises research entitled "Evaluation of methods for determining heavy metal content in polyethylene terephthalate food packaging" which considers and evaluates previously published methods used for the quantification of elemental composition in plastics used for food contact. The third chapter of this thesis is research entitled "Predictive Model for Online X-ray Fluorescence (XRF) Analysis of Antimony Content in Extruded Polyethylene Terephthalate Food Packaging" which seeks to develop a corrective equation for evaluating elemental contamination in plastics that would typically be too thin for analysis using XRF. The results of these research projects are prepared for publication in the *Journal of Plastic Film & Sheeting* and *Talanta*, respectively. Chapter 4 summarizes the findings of chapters 2 and 3 and offers suggestions for future research that builds off the findings herein. Appendix A provides additional figures that are supplemental to the research findings in Chapter 2. Appendix B gives a raw data table associated with the findings in Chapter 3.

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CHAPTER 2: EVALUATION OF METHODS FOR DETERMINING HEAVY METAL CONTENT IN POLYETHYLENE TEREPHTHALATE FOOD PACKAGING

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Abstract

Increased consumer awareness of heavy metal content in virgin and post-consumer recycled polymers for direct food-contact packaging has necessitated the development of analytical methods that identify and quantify heavy metals. Two common acid digestion methods produced incomplete sample digestion of polyethylene terephthalate (PET), thus, additional methods are required for proper analysis of PET. This study developed two modified microwave-assisted acid digestion methods resulting in complete PET digestion, which subsequently produced visually clear solutions. Inductively coupled plasma-optical emission spectrometry analysis of the completely digested PET resulted in heavy metal content values statistically higher concentrations of lead and antimony than for the methods that did not completely digest the PET polymer. The results of this study indicated that previously published research results might have unintentionally created bias toward lower heavy metal contamination in polymers used for food packaging. This is of concern when considering end-of-life disposal for food packaging with regulatory threshold levels for specific and total heavy metal content.

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Introduction

Increased consumer awareness of contamination in food-contact packaging has raised global concerns due to potential environmental contamination from packaging when disposed after the service lifetime [1]. Currently, several analytical methods are used to quantify consumer and environmental exposure to heavy metals stemming from the use of polymeric materials. Of importance is the safety of polyethylene terephthalate (PET), a polymer used extensively for direct food-contact packaging. In 2015, of the 5,971 million pounds of PET produced in the United States, 1,797 million pounds was recycled (30% recycling rate) [2]. Post-consumer polyethylene terephthalate (i.e., recycled; RPET) can be re-introduced into the supply stream for use in beverage bottles, thermoformed food containers, as well as a variety of non-food contact applications such as fiber [2]. Safety concerns surrounding the use of diverted plastic waste for consumer products led to the of introduction of legislation in 1992, set forth by the Coalition of Northeastern Governors (CONEG), which seeks to eliminate or reduce the amount of heavy metals lead, cadmium, mercury, and hexavalent chromium in packaging materials [3]. The CONEG model legislation was adopted in 1994 under the European Union's Packaging and Packaging Waste Directive (94/62/EC) [4]. Both CONEG and the 94/62/EC state that the total sum of lead (Pb), cadmium (Cd), mercury (Hg), and hexavalent chromium (Cr(VI)) shall not exceed 100 ppm.

Currently, there is no consensus on sample preparation methodology used to digest and analyze polymeric food-contact packaging for heavy metal content to comply with the legislation set forth by CONEG and 94/62/EC. Digestion of plastic matrices is necessary to degrade the organic material into an aqueous solution releasing the inorganic elements in the polymer matrix. Several literature methods of sample preparation are implemented and involve

the use of strong acids such as, sulfuric (H_2SO_4), nitric (HNO_3), and/or hydrochloric (HCl) acids, singularly or in combination with microwave radiation [5-13].

Takahashi *et al*. [5] determined the total antimony (Sb) concentration in PET via an elevated temperature acid digestion protocol. Antimony is a common catalyst for synthesizing virgin PET polymer resin [6, 14]. In the original Takahashi method, several samples from PET bottles were collected and placed in 18 M sulfuric acid for up to 4 h or until solubilized, but not fully digested, then heated by hot plate to 280 °C for 12 h to digest the polymer [5]. The H_2SO_4 method is effective as sulfuric acid has the ability hydrolyze PET after 4 hours at room temperature prior to the digestion step.

Microwave-assisted digestion (MWD) protocols have been utilized in the food, beverage, and material industries for the last four decades [15]. Prior to the development of MWD methods, conventional wet-ashing methods, such as those described by Brandão *et al.* and Vollrath *et al,* were used [8, 13]. Conventional wet-ashing requires continuous monitoring to avoid sample loss, special hoods for perchloric acid protocols, greater acid volumes, and often requires several hours [8, 13]. Microwave-assisted digestion for analysis of heavy metal content in plastics was widely adopted in the early 90s and is still the most common digestion method today [6, 7, 10, 11, 13, 16, 17]. Historically, MWD methods report the time and power parameters [10, 12, 13, 17-19], but not a controlled temperature ramp [6, 7, 11]. Advances in MWD technology provide the ability to control time, temperature, and pressure profiles [6, 7, 11].

One of the most widely cited PET methods is the Westerhoff microwave digestion method [5, 7-11, 16, 20-22]. Westerhoff *et al*. [6] described the digestion of PET from water bottles utilizing a HNO_3/HCl acid solution at 180 °C for 15 min and 250 psi. Nitric acid is

commonly used due to both its ability to degrade solid samples and for its low interference and background affects relative to other acids. HCl was added to HNO_3 due to the synergistic interactions that result increasing the digestion efficiency over HNO_3 alone.

When using the digestion methods described above, little consideration has been given to understanding the effect of the digestion method on the heavy metal concentrations obtained for PET samples for quantification with inductively coupled plasma (ICP) instrumentation. Though several studies have evaluated MWD methods by comparing MWD protocols to other methods such as ashing or microwave-induced combustion [8, 13, 19, 23], very few mention the presence of undigested particulates resulting from lower temperature digestions of PET. Takahashi *et al*. [5] addressed the issue of residual material resulting from low-temperature microwave digestion methods and concluded that a room temperature sulfuric acid digestion was sufficient for complete digestion of PET, however this was not realized in the current study. To assure the complete dissolution of PET, Takahashi *et al.* added a hot plate heating step at 280 °C for 12 h. [5] Brandão *et al*. [8] mentioned the need for filtering MWD samples when following the method of Westerhoff *et al.* [6] A study by Fan *et al*. [24] also reported residual material when following a modified version of the Westerhoff method, which utilizes hot plate heating instead of a microwave digestion system. Neither Brandão *et al*., Westerhoff *et al*., nor Fan *et al.* explicitly described the effect of undigested polymer on the quantitative results, potentially leading to data that does not accurately represent the true elemental composition as the residual material was removed post-digestion and prior to analysis. Brandão *et al*. reported that Sb concentrations obtained from samples digested using the MWD method were significantly less than concentrations obtained using a conventional wet-ashing method, but did not offer an interpretation of why differences were observed. Brandão noted that a

conventional wet-ashing method should be viewed favorably as a less-expensive alternative to microwave digestion method as both yielded "acceptable" results for analysis of Sb content in PET.

Antimony has been analyzed extensively in PET due to its widespread use as a polymerization catalyst. Although lead, cadmium, and chromium have been investigated less frequently, Pereira *et al*. [19] reported levels of Cd and Pb below the limit of detection (LOD) of 0.015 and 0.025 mg/kg, respectively. Perring *et al*. [23] compared the results of ICP-mass spectrometry (ICP-MS) to ICP-optical emission spectrometry (ICP-OES) for the quantification of Pb, Cd, and Cr using both conventional wet-ashing and microwave digestion techniques. Perring *et al.* [23] reported concentrations of 0.15, 0.02, and 16.0 mg/kg, for Pb, Cd, and Cr, respectively, when analysis was completed with ICP-MS. Analysis by ICP-OES yielded concentrations of 12.5 mg/kg of Cr in PET. [23] Levels of Cd and Pb were not quantifiable by ICP-OES, as they were below the LOD for the instrument. [23] Additionally, Curtzwiler *et al*. [25] reported Pb and Cd levels below the LOD (0.005 mg/kg; both Pb and Cd) in blends of virgin and recycled PET, but chromium was determined to be within the range of 5-31 mg/kg, depending on the recycled PET concentration. The current study builds on these findings by comparing methods commonly cited in current literature to digest PET and evaluate the Pb, Cd, and Cr, and Sb concentrations.

This study evaluated the effect of currently accepted sample preparation methods of PET for ICP quantification via microwave digestion. The temperature profiles and acid mixtures required for complete PET digestion and the effect of filtering undigested polymer particulates on the values of reported data were evaluated. Results of this study show that there are significant differences in concentration of both lead and antimony when different sample

preparation methods were used. If the concentration of a sample is near the threshold level (100 mg/kg sum of Pb, Cd, Cr(VI), and Hg), the choice of sample preparation method is expected to more strongly influence the results of regulatory compliance than previously believed.

Materials and methods

Sample Preparation

Polyethylene terephthalate (PET) polymer pellets were obtained from commercial suppliers on the West Coast and from the mid-western United States. Virgin, or first pass PET resin (8.1% crystallinity) and 100% post-consumer recycled (PCR) PET solid-state resin (6.5% crystallinity) was used. Virgin and solid-state PET pellets were blended to produce 0, 20, 40, 60, 80 or 100 % (wt/wt) PCR PET to evaluate the reliability of the digestion methods on various blends of PCR content. Each sample was cut with acid-cleaned (2% HNO_3; trace-metal grade) razor blades to reduce the potential for contamination.

Experimental design

Three total replications (n=3) of each digestion method and ICP-OES analysis were completed. The total analysis included six unique blends of PET (0-100% RPET) with three repeated measures per type. There were a total 18 samples per method (N=18) for a total of 54 observations for each method.

Standard Westerhoff Digestion

Nitric-hydrochloric acid combination digestions were completed following the method of Westerhoff *et al*. [6] Samples (250 mg) of virgin or blended PET were placed separately into 50 mL Teflon digestion vessels (Anton Paar, Graz, Austria). Each vessel received 10 mL of 15.7 M trace-metal-grade HNO_3 and 2 mL of 12.1 M trace-metal-grade HCl (Fisher

Scientific, Fair Lawn, NJ). Samples were digested using an Anton Paar Multiwave GO microwave digestion system (Anton Paar, Graz, Austria), which possessed a similar equipment setup as literature examples, at 180 °C and approximately 250 psi for 15 min, followed by a 10 min cooling cycle. The resulting digestions were diluted to a 50 mL final volume with ultra-pure, deionized 18.2 mega-Ohm (MΩ) water (Barnstead Genpure, Thermo Fisher Scientific, Waltham, MD). Diluted samples were filtered through an 18.2 MΩ water-rinsed Whatman No. 40 filter to remove undigested particulate matter.

Modified Takahashi Digestion

The method described by Takahashi *et al*. utilizes a relatively small sample size (60 mg) compared to other methods for ICP-OES analysis. As this may hinder the analysis, we increased the sample size, acid volume, and altered the heating protocol of the Takahashi *et al.* method. [5] 200 mg samples of virgin or blended PET were placed into 20 mL scintillation vials with 3 mL of 18 M H_2SO_4 (Certified ACS, Fisher Science Education, Nazareth, PA) and covered for 3-4 hours, or until all PET visually solubilized. Afterwards, 6 mL of 15.7 M HNO_3 was added to each vial followed by a 10 min ambient conditioning to allow exothermic reactions to subside. Dissolved samples were subsequently added to 18 mL disposable borosilicate glass tubes (Milestone Inc., Shelton, CT). Distilled water (150 mL) and 15.7 M HNO_3 (3 mL) were charged into the main reaction chamber for even heat distribution across the samples followed by loading the samples and sealing the chamber. The chamber was pre-pressurized to 580 psi (40 bar) with nitrogen and the samples were digested using a Milestone UltraWAVE digestion system (Milestone Inc., Shelton, CT) held at 210 °C for 20 min at approximately 725 psi (50 bar), followed by a 15 min cooling cycle to 60 °C. The final digested

solutions were clear, requiring no further sample preparation, and diluted to a 50 mL final volume with 2% HNO_3.

Modified Westerhoff Digestion

The method described by Westerhoff *et al.*[6] was modified by increasing the temperature profile from 180 to 260 °C in order to obtain complete digestion. Specimens of virgin and blended PET samples (125 mg) were digested in 18 mL disposable, borosilicate glass tubes. Each digestion tube received 5 mL of 15.7 M HNO_3 and 1 mL of 12.1 M HCl. Distilled water (150 mL) and 15.7 M HNO_3 (5 mL) were charged into the main reaction chamber for even heat distribution across the samples followed by loading the samples and sealing the chamber. After the samples were loaded and the chamber sealed, it was pre-pressurized to 580 psi (40 bar) with nitrogen. Samples were digested using a Milestone UltraWAVE digestion system held at approximately 1450 psi (100 bar) and 260 °C for 20 min, followed by a 10 min cooling cycle to 60 °C. The resulting digestions were clear, requiring no further preparation, and were diluted to 50 mL final volume with ultra-pure deionized 18.2 MΩ water.

Heavy Metals Analysis

A Thermo Scientific iCAP 7400 Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) DUO (Table 1) was used for the analysis of the lead (Pb), cadmium (Cd), chromium (Cr), and antimony (Sb) concentrations in each of the PET samples. Multi-element standards were diluted from 1000 µg/mL single standard solutions (Inorganic Ventures, Christiansburg, VA). Dilutions of the standard solutions were produced ranging from 0.010 µg/mL to 100 µg/mL. Yttrium (5 µg/mL) was used as an internal standard to correct for instrumental drift and to account for any variation which might occur due to differences in

acid composition between standards and samples. All samples were run with concurrent blanks which were solutions treated to the same digestion protocols but to which no PET had been added. This accounted for heavy metals present in the acids or leached from the reaction vessels, etc.

Table 1. iCAP 7400 ICP-OES DUO instrumental conditions and method parameters

Parameter	Operation Setting
RF Power Setting	1150 W
Pump Speed	50 RPM
Plasma Gas Flow	15 L/min
Auxiliary Gas Flow	0.5 L/min
Nebulizer Gas Flow	0.75 L/min
Sample Uptake Rate	1.5 L/min
Exposure Time	20s (UV) – 7s (Vis)
Wavelength (Pb)	220.353 nm
Wavelength (Sb)	217.581 nm
Wavelength (Cd)	226.502 nm
Wavelength (Cr)	284.325 nm

Statistical Analysis

JMP Pro 12 statistical software was used to analyze ICP results using a one-way analysis of variance (ANOVA). Means were separated by percent recycled content in PET using Tukey's Honestly Significant Difference (HSD) test.

Results

The original method of Westerhoff *et al*. yielded samples with visually high amounts of particulates. To increase the efficacy of Westerhoff protocol, a different microwave digestion system was utilized and the temperature protocol was increased from 180 °C to 260 °C. The increase in temperature produced visually clear digestion solutions after dilution. The

modified Takahashi and modified Westerhoff methods both resulted in complete digestions, visually free of any white particulates after dilution (Figure 1). The original Westerhoff method resulted in digestions with particulate material remaining (Figure 2), which was filtered prior to analysis. Microwave-assisted digestion methods have been evaluated in previous studies [8, 13, 19]; however, in these studies, there was no evaluation of the effect of filtering on the quantification of heavy metal content in the polymers investigated.

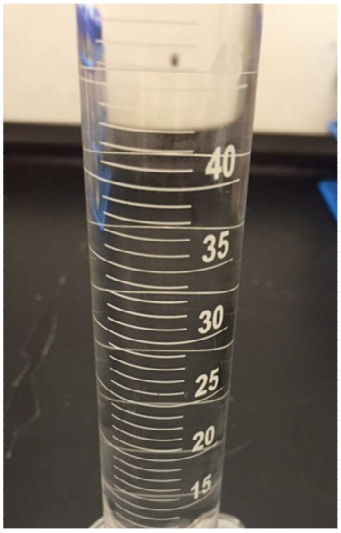

Figure 1. Digestion of PET resulting from the modification of the method of Takahashi *et al.* [5] Notice that all particulates have been solubilized.

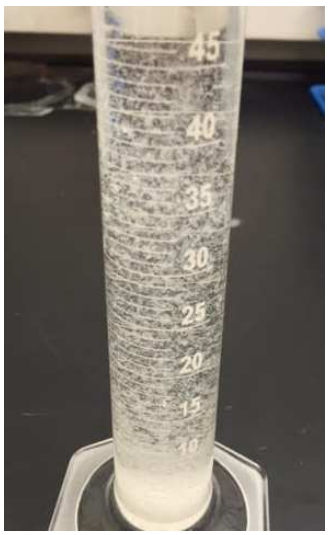

Figure 2. Digestion of PET using the original method of Westerhoff *et al.* [6] Notice the undigested particulates remaining at the end of the procedure.

Typical analysis of the Coalition of Northeast Governors' (CONEG) heavy metals includes: mercury, lead, cadmium, and hexavalent chromium [3]. Due to limitations in instrumentation, speciation of the two more common oxidation states of chromium (i.e. hexavalent and trivalent) were not discerned and only the total chromium concentration was quantified in the current study [26, 27]. Furthermore, while Sb is not within the scope of CONEG testing Sb was analyzed in the current study due to its prevalence in PET.

Takahashi *et al*. [5] noted the ability of sulfuric acid to completely digest PET at room temperature, however in the current study, complete digestion was not achieved without the addition of heat (210 °C). Sulfuric acid was shown to be effective at dissolving PET in approximately 3-4 hours; however, upon dilution, a cloudy solution was observed which suggests that the polymer may have solubilized then precipitated out of solution after dilution.

Since there are safety concerns associated with hot plate heating of strong acids and alternative elevated-temperature digestion instrumentation [6, 7, 10-13, 16-18] is available, a modified version of the Takahashi technique was developed in which PET was pretreated with H_2SO_4 without the use of heat. The protocol was modified by incorporating a microwave digestion step which yielded clear digestates after dilution which were free from particulate material requiring no filtering prior to ICP analysis.

Limit of detection and quantification

The detection capability of the ICP-OES was calculated as three times the standard deviation of the reagent blank, divided by the slope of the calibration curve for the limit of detection (LOD). The limit of quantification (LOQ) was calculated as 10 times the standard deviation, divided by the slope of the calibration curve. The values for LOD and LOQ are displayed in Table 2 for each element at their respective wavelengths for ICP-OES analysis.

Table 2. Detection (LOD) and quantification limit (LOQ) for Pb, Cd, Cr, and Sb

Element	Wavelength (nm)	LOD (µg/kg)	LOQ (µg/kg)
Pb	220.353	2.76	9.20
Sb	217.581	5.13	17.10
Cd	226.502	0.33	1.53
Cr	284.325	3.47	11.56

Spike recoveries

The recovery analysis was carried out using three different concentrations (1, 10, & 100 µg/mL) for each method and the results are given in Table 3. The percent recoveries for both the standard Westerhoff and modified Westerhoff methods were within the acceptable limits (80 - 120%) for all elements as set forth in EPA Method 6010D [28]. However, the percent recovery of Cd for both versions of the Westerhoff method were very close (119% of

spiked concentrations) to being outside the range of acceptability. The modified Takahashi method was outside the range of acceptability for two of the three Pb concentrations and one of the Sb concentrations. These results indicate that the modified Takahashi method may significantly under-report Pb concentrations in PET at concentrations below 10 mg/L (ppm).

Table 3. Percent Recovery of 1, 10, and 100 mg/L Pb, Cd, Cr, and Sb from spiked samples for each digestion method.

Heavy Metals	Modified Westerhoff			Modified Takahashi			Standard Westerhoff		
	1 mg/L	10 mg/L	100 mg/L	1 mg/L	10 mg/L	100 mg/L	1 mg/L	10 mg/L	100 mg/L
Pb	90%	96%	94%	76%	79%	84%	89%	92%	98%
Sb	97%	101%	100%	75%	84%	88%	93%	93%	96%
Cd	113%	119%	116%	98%	101%	103%	119%	116%	114%
Cr	83%	90%	91%	85%	87%	91%	95%	93%	99%

Antimony

Samples analyzed for Sb using the standard Westerhoff digestion method consistently yielded concentrations that were significantly higher ($p < 0.05$; Figure 3) than samples analyzed using the modified Takahashi for all samples except for 80% and 100% RPET. Antimony concentrations were significantly higher for samples digested using the modified Westerhoff method for 100% virgin and 100% PCR PET, however, all other samples were not significantly different in antimony concentration as determined via Tukey's HSD test ($\alpha=0.05$). The lower reported of Sb concentrations by the modified Takahashi method could be the result of losses due to sample preparation, as indicated by the percent recovery analysis. Previous studies have reported Sb concentrations, in Sb catalyzed PET, ranged from

150-300 mg/kg [6, 7, 10, 11]. For all three digestion methods evaluated here, the Sb concentration was within the previously reported range.

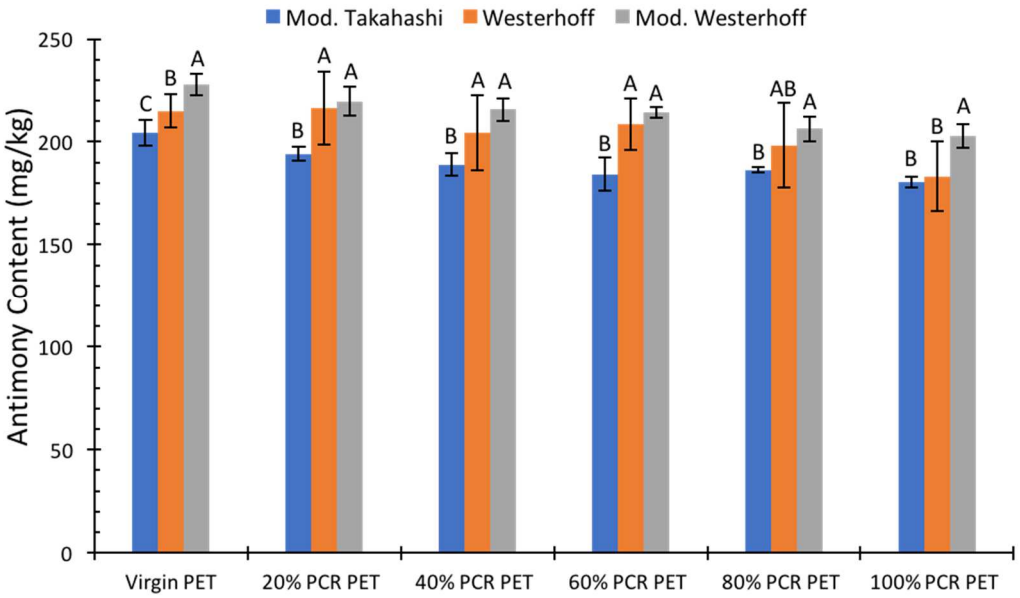

Figure 3. Antimony levels, for blends of virgin and recycled PET (n=3, N=18; 54 total observations per method). Tukey's letters of significance (A, AB, B, and C) are presented over the error bars. Letters that are not the same indicate significant differences between methods by Tukey's HSD test for that blend of PET.

Chromium

All sample preparation methods yielded concentrations of total chromium which were not statistically different, ($p > 0.05$; Figure 4). Significant differences were not observed due to the large variation in determined concentrations of chromium across all sample types. Virgin PET, 60% PCR PET, and 80% PCR PET showed a large separation of means, but sample error negated these differences. Mean concentrations were well above the LOQ (0.02 mg/kg), thus it would not be expected that low detection limits would be the cause of the large sample error. Previous studies reported Cr concentrations in the range of 5-31 mg/kg. Mean concentration levels of Cr in PET for the current study were found to be 0.3 – 0.9 mg/kg. Spike analyses

suggest that all methods under-reported Cr concentrations, this could be an explanation for lower observed values. Additionally, compared to previous studies, the lower Cr levels could be explained by variation based on resin source or perhaps a method was used which over-reported Cr concentrations in PET.

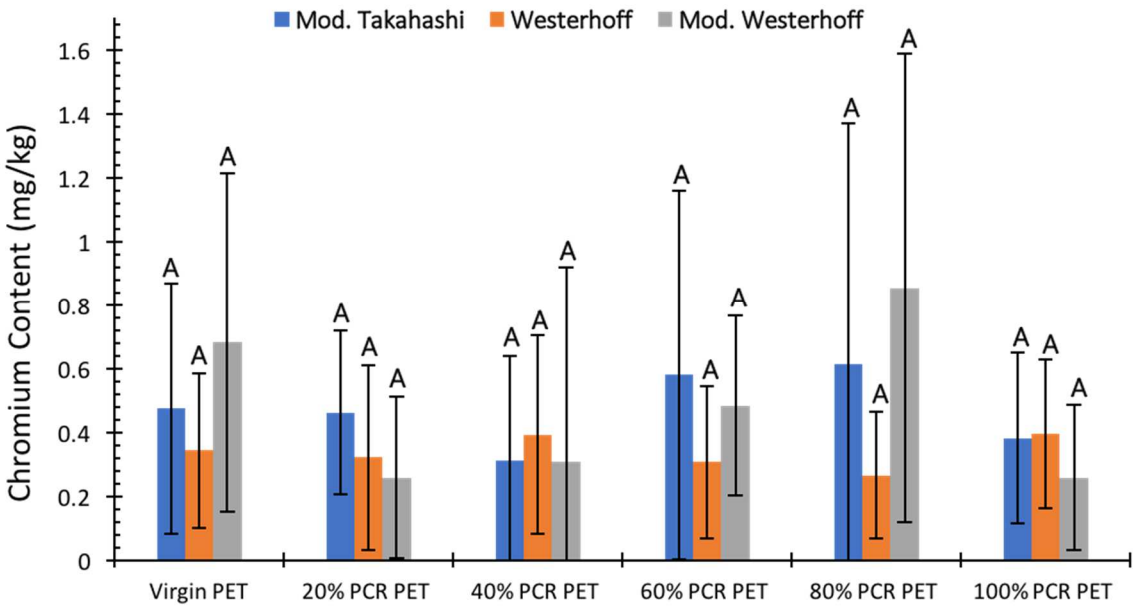

Figure 4. Chromium levels, for blends of virgin and recycled PET (n=3, N=18; 54 total observations per method). Tukey's letters of significance (A) are presented over the error bars. Letters that are the same indicate no significant differences between methods, according to Tukey's HSD test.

Cadmium

All sample preparation methods yielded concentrations of cadmium which were not statistically different, ($p > 0.05$; Figure 5). Concentrations of cadmium obtained from Virgin PET samples were much higher than seen in the other blends, but were not different from one another. The low concentrations of Cd in PET may result in variation due to quantification levels of ICP-OES. Studies which were able to quantify Cd in PET determined concentrations of approximately 0.02 mg/kg [23]. The average concentration from the current study of Cd in PET was determined to be in the range of 0.01 – 0.1

mg/kg. The average concentration from the current study of Cd in PET was determined to be in the range of 0.01 – 0.1 mg/kg. Higher than expected concentrations were observed in virgin and 20% RPET samples across all three methods. These high levels, along with a steep decline from virgin PET to 20% RPET may have been the result of sample homogeneity or variation within the sample set. It would be expected that if virgin PET was very high and 100% RPET was quite low, that a slow decline with even distribution would be observed. Spike analyses suggest that the standard and modified Westerhoff methods over-reported Cd concentrations, this could be an explanation for higher observed values.

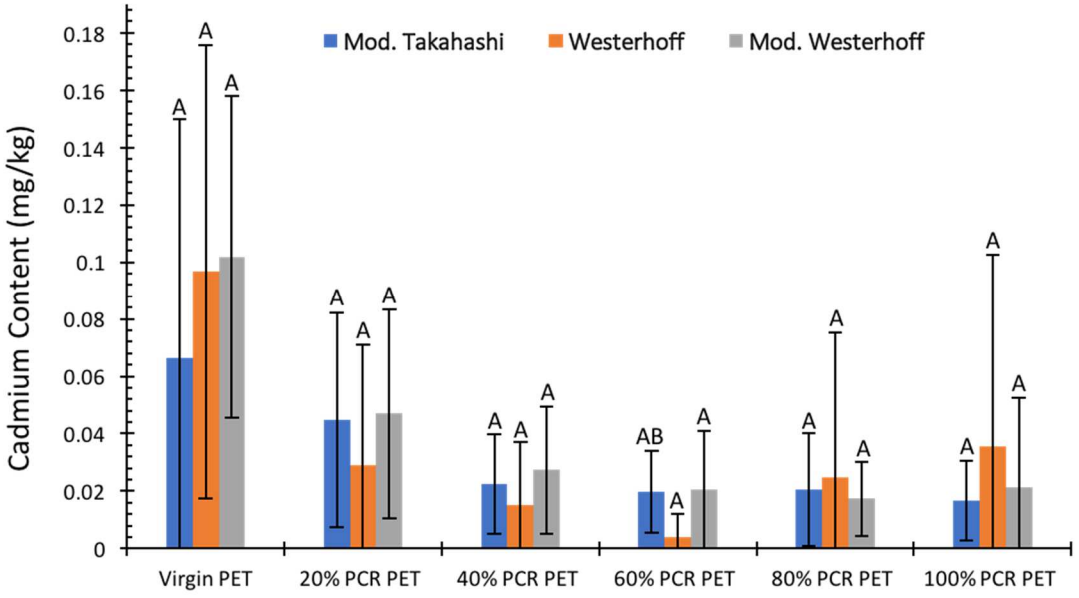

Figure 5. Cadmium levels, for blends of virgin and recycled PET (n=3, N=18; 54 total observations per method). Tukey's letters of significance (A, AB) are presented over the error bars. Letters that are the same indicate no significant differences between methods, according to Tukey's HSD test.

Lead

Considerably higher concentrations of lead were observed when samples were digested using the modified Westerhoff method and were significantly different ($p < 0.05$; Figure 6) from the standard Westerhoff methods for all samples and from the modified Takahashi method for

virgin and 80% PCR PET. No differences for lead concentration were observed between the modified Takahashi and standard Westerhoff methods. Previous analysis of Pb in PET was reported by Perring *et al.* to be 0.15 mg/kg [23]. Average concentrations in the present study determined that Pb concentrations ranged from 0.06 – 0.7 mg/kg.

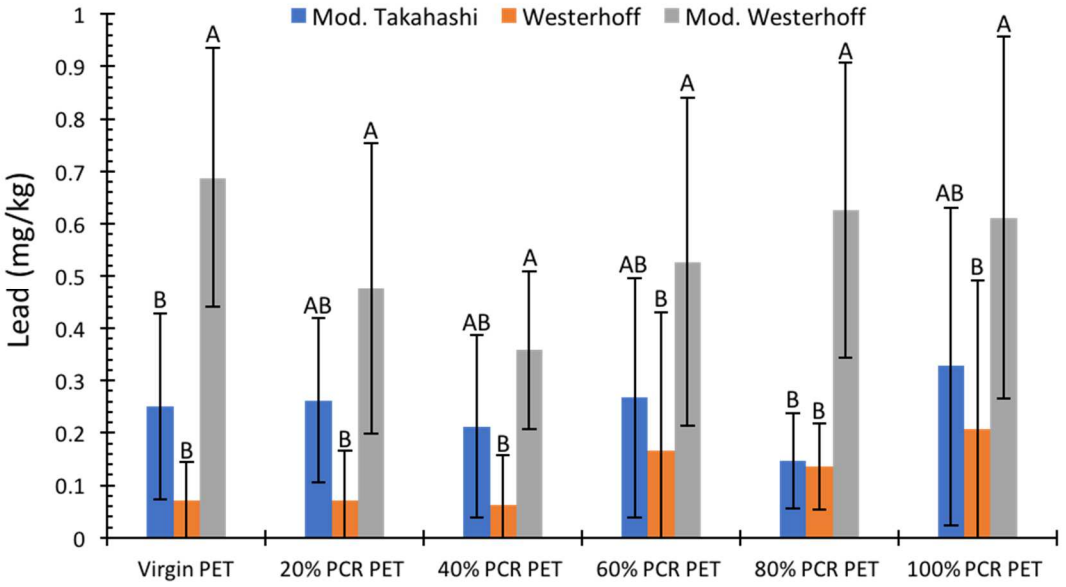

Figure 6. Lead levels, for blends of virgin and recycled PET (n=3, N=18; 54 total observations per method). Tukey's letters of significance (A, AB, B) are presented over the error bars. Letters that are not the same are significantly different, according to Tukey's HSD test.

CONEG analysis

Toxics in packaging requirements state that the sum of heavy metal content (Pb, Cd, Cr, and Hg) is not to exceed 100 mg/kg in polymeric packaging materials. Figure 7 provides an example of the sum of the metals found in virgin PET from the three methods analyzed in this study. Mercury was not analyzed in the present study due to lack of instrumentation for the ICP-OES system (i.e. cold vapor attachment). The remainder of the heavy metal sums, as they relate to CONEG, can be found in the Appendix A (Figures A1-A5). The sum of the three heavy metals analyzed range from 0.5 -1.4 mg/kg for virgin PET. The standard Westerhoff

method which used a lower temperature digestion and required filtering consistently yielded total sums which were lower than those obtained from the modified Takahashi and modified Westerhoff methods. In addition, the modified Takahashi method consistently yielded sums which were below the level obtained from the modified Westerhoff method.

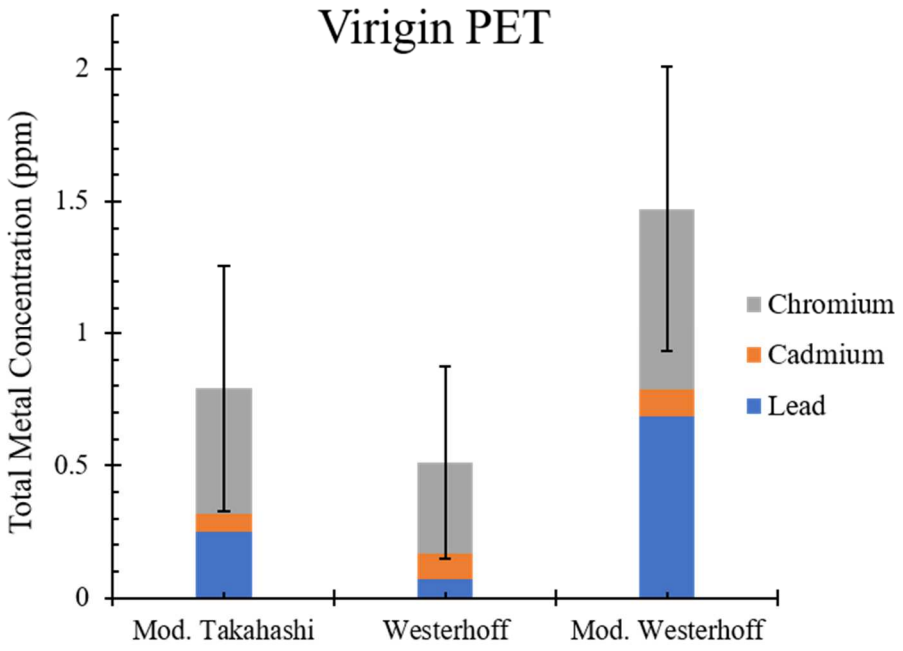

Figure 7. Sum of CONEG heavy metals for virgin PET, digested using three methods. Error bars represent the sum of heavy metal standard deviations, for each method.

Discussion

There was a total of 18 samples per method (N=18) and each method was replicated three times (n=3). The method described by Westerhoff *et al.* [6] yielded digestions with visibly high amounts of particulate remaining while the modified Westerhoff and modified Takahashi methods yielded digestions which were visibly clear. Higher average concentrations (Figures 3 & 6) of Sb and Pb were found in samples digested using the modified Westerhoff method when compared to samples digested using the standard Westerhoff and modified Takahashi methods. Slight variations can be attributed to the inherent variation observed in

heavy metals analysis. However, it is possible significant differences could be attributed to filtration. The initial sample mass is utilized to calculate the concentration of heavy metals present in the final solution. When a significant amount of material remains undigested and is filtered after dilution, the result is skewed and the calculated concentration reduced. The effect of filtration on the total sum of regulated heavy metals (Figure 7) was such that the standard Westerhoff method was three-times lower than the total sum as determined by the modified Westerhoff method in virgin PET.

The Toxics in Packaging Clearinghouse (TPCH) was established by CONEG to regulate and certify manufacturers for compliance of the heavy metals legislation. The TPCH states that sample preparation methods used for the purposes of certification must achieve complete digestion of the sample as the metals present would not be completely liberated from the material and thus cannot be accurately measured [29, 30]. However, the TPCH cites Environmental Protection Agency (EPA) Method 3052 as a method that is sufficient for the complete dissolution of plastics [30]. EPA Method 3052 is written for the decomposition of organic and siliceous materials and is similar in design to that of Westerhoff *et al.* [6], i.e., a low-temperature digested is used, which resulted in an incomplete digestion of PET (Figure 2). A low-temperature digestion method is not recommended to ensure accurate reporting of total heavy metal content in PET as our data demonstrates a consistent under reporting of the calculated concentrations.

Accuracy of heavy metals quantification is commonly carried out using a certified reference material of the same matrix as the unknown sample [7, 19, 23, 24]. As certified PET reference materials are not available, spiked samples were used to evaluate digestions of known concentration. The modified Takahashi method was the only method to yield results

outside the range of acceptability (±20%) for Pb and Sb. This study directly measured and determined significant differences in the quantified concentrations of the heavy metals regulated by CONEG, however the total heavy metal concentration of each PET/RPET blend was far below the CONEG threshold limits for the samples investigated here. It was shown that filtering particulates of undigested PET significantly reduced the measured concentration of lead and antimony, which can potentially mislead packaging safety assessors when considering a packaging structure to satisfy specific regulations.

Conclusions

PET resin samples containing 0-100% RPET were analyzed via ICP-OES to investigate the effect of the digestion protocol on the reported concentration values of lead, cadmium, chromium, and antimony. The results indicated that the values obtained via ICP-OES for Pb and Sb contamination in virgin and recycled PET are affected by the sample preparation protocol, while Cd and Cr appear to be unaffected by the choice of sample preparation method. The reasoning why differences were observed in Pb and Sb, but not in Cd and Cr, is not clear and is a topic of current investigations. The large variability observed in the quantification of Cr centers around the capability of equipment used for analysis.

The objective of this study was to observe the effect of sample preparation method as well show the inherent variation that occurs with ICP-OES testing of CONEG-regulated heavy metals. Depending on the sample preparation method selected, food packaging materials may fail regulatory thresholds for safety (as set forth by the CONEG legislation on toxics in packaging and the European Union's Packaging and Packaging Waste Directive 94/62/EC) or be falsely reported as compliant when the overall metal content is higher than the regulated threshold levels. All samples analyzed for total heavy metal content in this study were well

below the threshold (100 mg/kg) level set forth by CONEG and were in the range of 0.4 – 1.5 mg/kg across all virgin and recycled PET samples. These results have presented concerns with regards to the accuracy of previously reported heavy metals analysis if higher than usual contamination is observed. In addition, if future compliance levels are decreased, the accuracy of heavy metals quantification and reporting becomes increasingly important.

The modified Westerhoff method, which consistently yielded complete digestion of the polymer, regularly reported higher concentrations for lead and antimony when compared with the other two methods. As such, we recommend that a method comparable to the modified Westerhoff method be applied for the digestion and analysis of heavy metal concentrations in PET polymer matrices.

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CHAPTER 3: PREDICTIVE MODEL FOR ONLINE X-RAY FLUORESCENCE (XRF) ANALYSIS OF ANTIMONY CONTENT IN EXTRUDED POLYETHYLENE TEREPHTHALATE FOOD PACKAGING

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Abstract

Heavy metal contaminants in food-contact plastics have the potential to cause health issues if leaching were to occur. Regulations surrounding heavy metals in these materials seeks to reduce these levels by holding manufacturers accountable. However, traditional analytical methods such as inductively coupled plasma (ICP) and atomic absorption spectroscopy are time-consuming and expensive processes. X-ray fluorescence (XRF) technology provides a means for monitoring heavy metals content, thereby greatly reducing the costs associated with testing by traditional methodology. However, a major downfall of XRF analysis is that a sample must be of sufficient thickness for reliable quantification of elements. The results of this study demonstrated the ability to analyze antimony concentrations in thin plastic samples below the infinite thickness by developing a correlation correction factor by varying sample thickness from XRF and ICP data. The current model accurately predicted ICP concentrations from XRF data for 94% of the samples tested.

Introduction

Regulation of toxic heavy metals in food packaging plastics has led to the development of qualitative and quantitative methods of analysis. High concentrations of heavy metals in

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food packaging materials are of concern because they have the potential to migrate into food substrates when stored under abuse conditions and become an environmental hazard after disposal [1, 2]. Concerns regarding the safety of food-contact materials resulted in the introduction of legislation both in the United States and abroad that regulates the total sum of the heavy metals lead, cadmium, hexavalent chromium, and mercury in packaging materials [3, 4]. While antimony (Sb) is not regulated in packaging materials in the United States, it is considered highly toxic and is regulated by the Environmental Protection Agency (EPA) to a maximum contaminant level of six μg/L (ppb) in drinking water [5]. Methods used for determining concentrations of heavy metals in packaging materials are time-consuming and expensive [4]. Traditional analysis of food-contact packaging involves sample decomposition/digestion by open or closed-vessel digestion [4, 6, 7]. Quantification of heavy metals can be carried out using several methods, two of which are atomic absorption spectroscopy (AAS) and inductively coupled plasma (ICP) [4, 6, 7]. The time required for heavy metal quantification using ICP or AAS is three to four hours for a small number of samples or a day or more for larger sample sets due to the need for sample preparation, decomposition, and subsequent analysis [4]. The lag time associated with this type of analysis could be detrimental if unusually high contamination levels are observed, as product recall may not be feasible. A more rapid, but generally less sensitive, analytical method for the quantification of heavy metal content is X-ray fluorescence (XRF) spectroscopy and its variants.

XRF is a widely accepted analytical technique for determining elemental concentrations in a variety of sample matrices such as painted surfaces, coal, soil, and plastics [8-12]. When sufficient energy is applied to the sample by incident X-ray, an inner-orbital

electron of an atom is ejected. An outer shell electron will fill the vacancy and emit a X-ray photon with an energy characteristic of a specific element [11]. Like ICP and AAS analysis, XRF is a comparative method meaning that standards of known concentration must be used to calibrate the instrument and provide a reference for unknown sample intensities. There are two types for XRF analyzers: wavelength dispersive XRF (WDXRF) and energy dispersive XRF (EDXRF). The WDXRF analyzer uses a diffraction crystal to separate X-rays according to wavelength. The EDXRF analyzer directs secondary X-rays to a detector that converts the X-ray into a voltage signal. WDXRF analyzers have a larger footprint, lower detection limits, but are more expensive than EDXRF analyzers. EDXRF has the ability quantify specific or multiple elements simultaneously. Analysis by all XRF instrumentation is non-destructive, requires very little sample preparation, and takes between one and three minutes to complete. In addition, once calibrated, XRF systems are capable of repeated analysis with minimal maintenance and only periodic recalibration. However, an inconvenience of XRF analysis is that a sample must be of sufficient thickness; the common term for this property is infinite thickness for reliable quantification of elements.

The term infinite thickness describes the depth that the primary X-ray must travel into and out of the sample [13]. A sample analyzed by XRF should be thicker than the depth that the X-rays can travel into a material as samples that are of less than infinite thickness are partially transparent to incident X-ray and sufficient excitation may not occur [13]. The calculation for infinite thickness is determined, in part, using the Beer-Lambert Law (Beer's Law; Eq. 1). The mass attenuation coefficient (MAC; Eq. 2) is obtained from experimentally and commonly from National Institute of Standards and Technology (NIST) Tables for each element [14]. In addition, the infinite thickness $(t_{inf}; \text{Eq. } 3)$ changes based on the density of a

given material [13]. Using equations 1-3, the infinite thickness for PET was determined (Eq. 4) to be 21 mm.

1. $I_f = I_0 e^{-\mu\rho d}$
2. $\mathrm{MAC} = \mu_{\mathrm{i,e}} * \mathrm{C_i} + \mu_{\mathrm{j,e}} * \mathrm{C_j} + \mu_{\mathrm{k,e}} * \mathrm{C_k}$
3. $\frac{1}{\mu\rho} = t_{inf}$
4. $\mu = 0.625_{\mu_{Sb-C}} + 0.042_{\mu_{Sb-H}} + 0.333_{\mu_{Sb-O}}$
   $(0.625)(0.28) + (0.042)(0.40)$
   $+(0.333)(0.47)$
   $= 0.348$
   $= \frac{1}{0.348*1.38} = 21 \text{ mm}$

PET: $C_{10}H_8O_4$
ρ= Density of PET: 1.38 g/cm^3
$\mu_{\mathrm{x,e}}$= NIST Table MAC of PET in cm^2/g

- Sb through Carbon = 0.28
- Sb through Hydrogen = 0.40
- Sb through Oxygen = 0.47

C_x= Concentration of "*i-k*", as a percent

- C: 12*10 = 120 or (62.5%)
- H: 1*8 = 8 or (4.2%)
- O: 16*4 = 64 or (33.3%)

Plastics commonly used for food contact packaging are between 0.3 and 0.6 mm thick. Thus, the sample thickness required to properly analyze PET is generally not obtained for food packaging applications, thus, a correction factor is required for accurate measurements. Analyzing samples that are thinner than infinite thickness results in is a lower counts-per-second (cps) or weaker intensity than would be observed from a sample that is of sufficient thickness. A weaker recorded intensity corresponds to a concentration output, which is lower than the actual concentration. Analysis for elemental content by XRF has been utilized in a variety of applications such as plastics, coal, wood pulp, cement and limestone, and chrome-iron ores [8, 9, 15, 16]. XRF has been utilized as an online application for determining elemental composition for some of these applications. When XRF is setup over continuous systems (i.e. online) such as on a conveyor belt, the analysis can be carried out so that the material is not disturbed or damaged. With the high overall cost and high analysis time of traditional heavy metal quantification methods (e.g., ICP and AAS methods), online XRF systems could play a vital role in monitoring extruded plastics for elemental content.

In the current study, the antimony (Sb) concentration in extruded PET sheet was determined by XRF and ICP-OES to develop a method that correlates antimony concentration

in PET. Antimony levels obtained from XRF were correlated with those obtained from ICP-OES to evaluate the response and develop a correction equation for samples that were not infinitely thick. According to EPA Method 6200, XRF outputs are considered acceptible if they accurately report concentrations for a certified reference material within a 20% margin of error [17]. Correlated data were used to predict Sb content in unknown PET samples and were considered accurate if with the 20% margin of error, as set forth in EPA Method 6200. The prediction accuracy of the model developed herein was 94% within a 20% margin of error.

Materials and Methods

Sample preparation

Material used to develop the predictive model was extruded, 18 mil (0.5 mm) PET sheet. Specimens were cut using acid cleaned scissors into 4 cm x 4 cm square coupons. PET coupons were thoroughly washed with ultra-pure deionized water (18.2 MΩ; Barnstead Genpure, Thermo Fisher Scientific, Waltham, MD) and dried (100 °C; 4 hr.). As displayed in Figure 1, stacks of PET samples were used to develop the predictive model. Total sample thickness ranged from 0.5 – 20 mm. However, individual PET samples of varying thicknesses (0.332 – 0.545 mm) were used to test the predictability of the model.

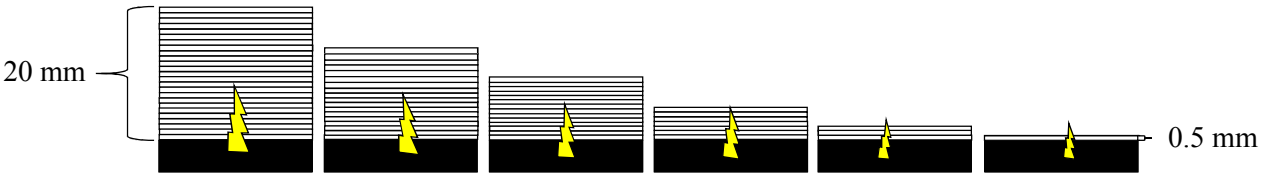

Figure 1. Schematic for the analysis of PET at varying sample thickness using EDXRF. Each sample stack was analyzed individually. Black boxes represent the analysis surface and the lightning is the incident X-ray

Energy dispersive X-ray Fluorescence analysis (EDXRF)

A NEX OL EDXRF analyzer (Applied Rigaku Technologies, Austin, TX) equipped with a 50 kV X-ray tube with maximum power of four Watts, 7.8-µm beryllium window, and

silicon drift detector (SDD) was utilized to analyze the PET samples. A series of 16, polyethylene National Institute of Standards and Technology (NIST)-traceable, ASI standards (Analytical Services Inc., Oak Ridge, TX), including a blank sample containing no traceable metals, were used to calibrate the EDXRF analyzer for Sb content in the range of 5 – 300 mg/kg. To evaluate the same lot of PET samples at varying thicknesses, multiple PET coupons were placed over the X-ray source at thicknesses ranging from 0.5 – 20.0 mm (Figure 1).

Microwave-assisted digestion

Samples of PET were digested using a Milestone UltraWAVE digestion system (Milestone Inc., Shelton, CT) held at approximately 1450 psi (100 bar) and 260°C for 20 minutes, followed by a 10-minute cooling cycle to 60 °C. Specimens (150 mg) were digested in 18 mL disposable, borosilicate glass tubes. Each digestion tube received trace-metal-grade 15.7M nitric acid (5 ml; HNO_3) and trace-metal-grade 12.1M hydrochloric acid (1 mL; HCl) (Fisher Scientific, Fair Lawn, NJ). Distilled water (150 mL; 18.2 MΩ) and 15.7M HNO_3 (5 mL) were charged into the main reaction chamber for even heat distribution across the samples followed by loading the samples and sealing the chamber. The chamber was pre-pressurized to 580 psi (40 bar) with nitrogen prior to digestion. The resulting digestions were clear, requiring no further preparation, and were diluted to final volume with ultra-pure deionized water (50 mL; 18.2 MΩ).

Inductively coupled plasma analysis

An iCAP 7400 ICP-OES DUO (Thermo Scientific, Waltham, MA) (Table 1) was used for the analysis of antimony (Sb) content in the PET samples. Sb standards were diluted from a 10,000 mg/L single standard solution (Inorganic Ventures, Christiansburg, VA). Dilutions ranging from 100 mg/L to 300 mg/L were used to establish a 5-point calibration curve. Yttrium

(5 mg/L) was used as an internal standard to correct for instrumental drift and to balance any variation that may occur due to differences in acid composition between standards and samples. All samples were run with concurrent blanks which were solutions treated to the same digestion protocols but to which no PET had been added.

Table 1. iCAP 7400 ICP-OES DUO instrumental conditions and method parameters

Parameter	Operation Setting
RF Power Setting	1150 W
Pump Speed	50 RPM
Plasma Gas Flow	15 L/min
Auxiliary Gas Flow	0.5 L/min
Nebulizer Gas Flow	0.75 L/min
Sample Uptake Rate	1.5 L/min
Exposure Time	20s (UV) – 7s (Vis)
Wavelength (Sb)	206.833 nm

Statistical analysis

R-studio statistical software was used to analyze and model the relationship between EDXRF and ICP Data for Sb content in PET by sample thickness.

Results and discussion

Limit of detection for EDXRF

Figure 2 shows the calibration curve for Sb, obtained by measuring 16 polyethylene standards ranging in concentration from 5 – 300 mg/kg and one blank polyethylene standard that contained no traceable metals. Excellent correlation was observed for the calibration with an R^2 value of 0.99.

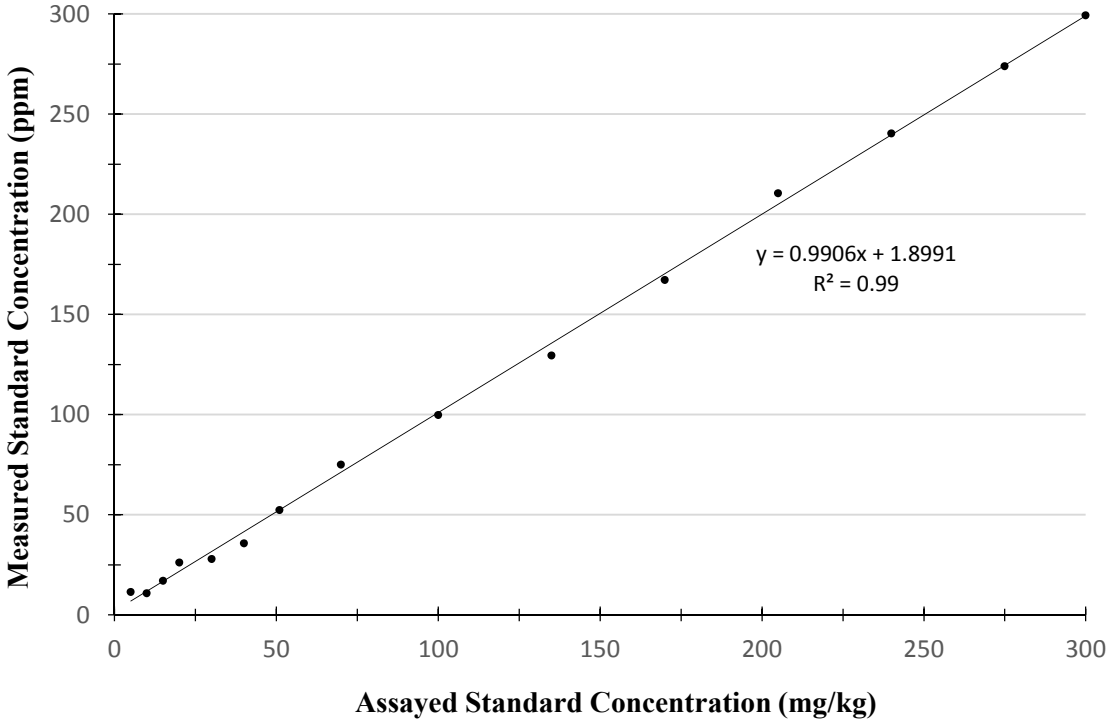

Figure 2. Calibration curve for Sb standards determined by EDXRF

Values for the limit of detection (LOD) and limit of quantification (LOQ) were calculated in accordance with IUPAC equations (Equations 4 and 5) [18]:

$$\mathrm{LOD} = \frac{K_D \times S_b}{q_1} \qquad (4)$$

$$\mathrm{LOQ} = \frac{K_Q \times S_b}{q_1} \qquad (5)$$

Where K_D and K_Q are constants and represent a separation of three and 10 times the standard deviation of repeated blank measurement, respectively [18]. S_b is the standard deviation of 10 blank measurements, and q_1 is the slope of the calibration curve [18]. The LOD and LOQ for this EDXRF were determined to be 4 mg/kg and 14 mg/kg, respectively. These values represent a range at which we can begin discerning differences in Sb concentration between two samples.

Precision and accuracy of EDXRF

To evaluate precision and accuracy of the EDXRF system, percent relative standard deviation (%RSD) and percent error (%D) were calculated using the equations 6 and 7, respectively [8]:

$$\%\mathrm{RSD} = \frac{\sigma}{\mathrm{X_{av}}} \times 100\% \qquad (6)$$

$$\%\mathrm{D} = \frac{|\mathrm{X_{av}} - \mathrm{X_{re}}|}{\mathrm{X_{re}}} \times 100\% \qquad (7)$$

Where σ is the standard deviation for the repeated measurements, X_{av} is the average value of the repeated measurements, and X_{re} is the reference value for Sb content, as determined by ICP-OES [8]. The %RSD and %D were determined to be 1.8% and 5.9%, respectively. A 1.8% RSD indicates a high level of precision and excellent agreement between XRF measurements under identical conditions. The value obtained for %D indicates how much the average concentration from repeated online XRF measurements for each element differed from the reference value. The value of 5.9% indicates that the measured values did not exactly match the reference sample, but measurements were well within the acceptable range of 20% [17].

Experimental results

This study proposed that a correction factor could be developed for XRF using thin PET samples that were stacked at varying thickness. Five different lots of PET sheet were used to determine the concentration of Sb at thicknesses ranging from 0.5 mm to 20 mm, in increments of 2.5 mm as noted in Figure 1. Average concentrations of Sb in each PET lot were obtained from ICP-OES and were correlated back to XRF responses. It was observed that the Sb concentration increased with thickness for the same lot of PET samples from approximately 6 to 200 mg/kg (Figure 3).

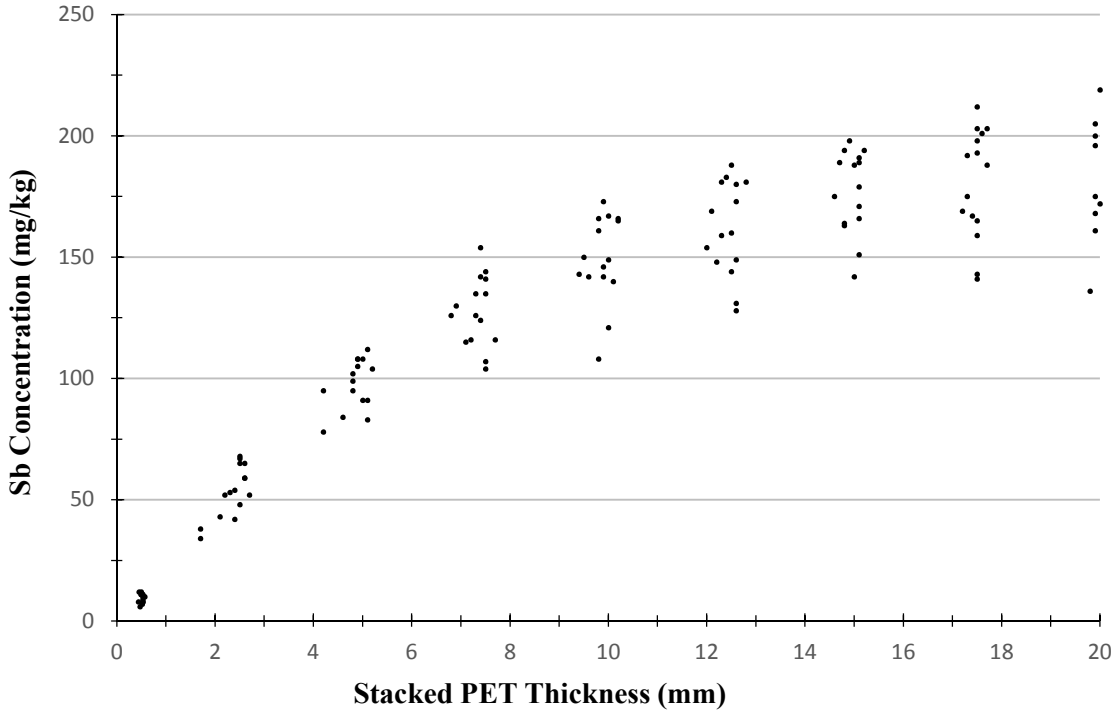

Figure 3. Concentrations of Sb, as determined by EDXRF, at varying thicknesses (N=5, n=10)

Concentrations of Sb were determined by ICP-OES and the ratio of XRF-to-ICP was plotted as a function of thickness. Values appear to asymptote at a ratio of one where the Sb concentration measured by XRF is equal to concentration measured via ICP-OES. It is expected that a properly calibrated XRF analyzer will accurately report concentrations at the infinite thickness of the material being tested, which in this case is PET with an infinite thickness of 21 mm. Using the data from Figure 4, a generalized nonlinear least square fit analysis was completed.

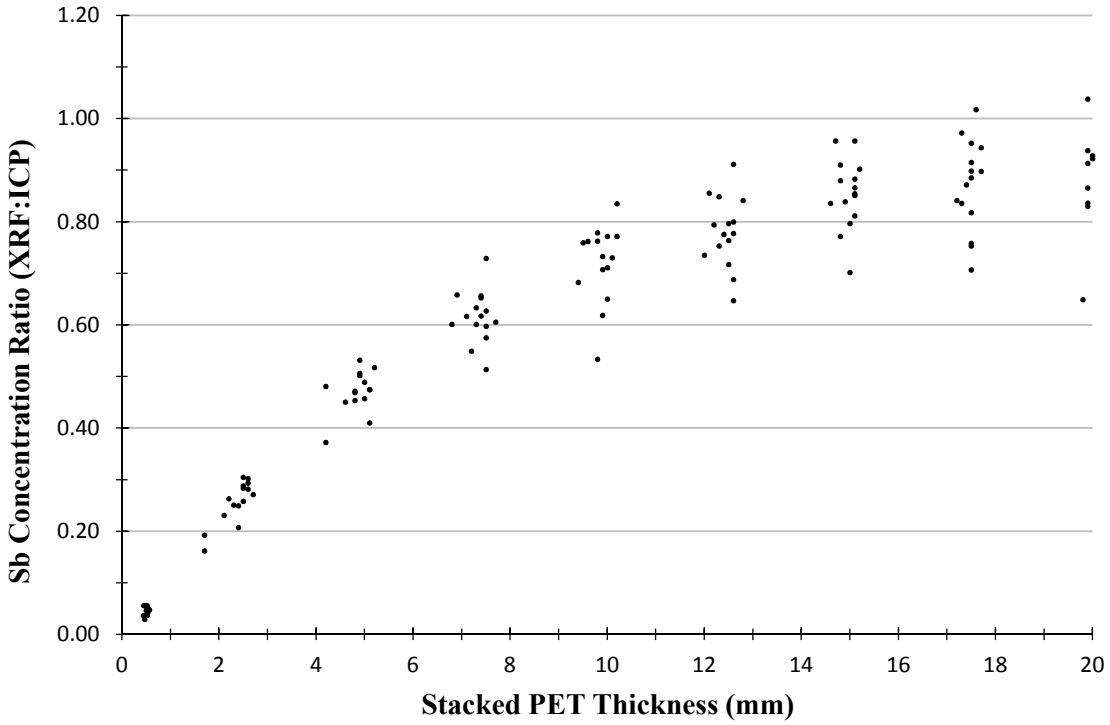

Figure 4. Ratio of XRF to ICP concentration of Sb, as recorded by XRF, for samples ranging from 0.5 – 20 mm thick (N=5, n=10)

The fitted model was used to develop an equation capable of providing a correction factor to adjust the Sb concentration of PET measured by XRF to the concentration measured by ICP-OES as a function of the sample thickness. An approximation of the equation is provided below (Eq. 8). Where d is the thickness and x and y are fitting parameters.

$$ICP_{predicted} = (XRF_{actual}) \times \left(\frac{1}{(x)\left(1 - e^{-\frac{d}{y}}\right)} \right) \qquad (8)$$

The equation was used to assess a potential range of ICP concentrations for nine unique PET samples with thicknesses ranging from 0.332 – 0.545 mm, which were different from materials used to make the equation. For this analysis, only one PET coupon was analyzed both by XRF and ICP per test. Each unique PET specimen was split into four samples (4 cm x

4 cm, each) and scanned with the XRF analyzer. Each of the four samples were then analyzed by ICP-OES. A 20% margin of error was utilized as a measure of accuracy for the corrective equation as was used in EPA Method 6200 [17]. Of the 36 XRF measurements, 34 were accurately corrected by the equation within a 20% margin of error (See Appendix B; Table B1 for the full data set). The data were averaged for the XRF corrected and actual ICP concentrations across all nine samples types (Figure 5). Individual values that were not accurately predicted were spliy between over-estimating and under-estimating the response. Further development of the equation may be necessary to account for these inaccuracies and to increase accuracy of prediciton rates at a lower margin of error.

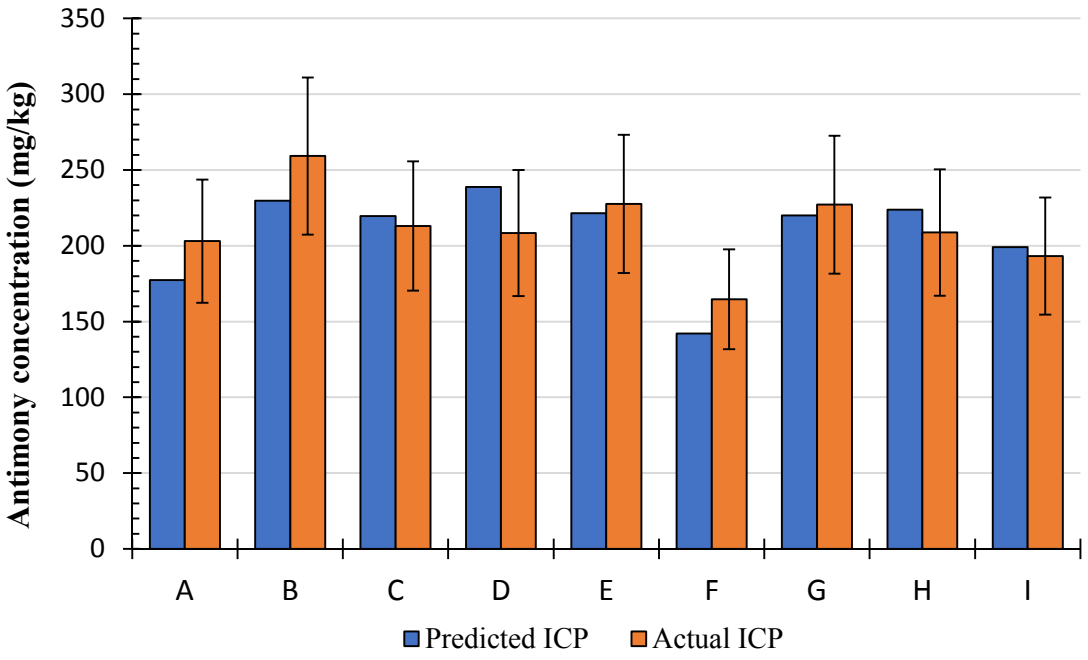

Figure 5. Predicted Sb concentrations, estimated from XRF values versus actual Sb concentrations as determined by ICP-OES. Error bars represent a 20% margin of error (N=9, n=36). Materials "*A-I*" represent nine unique PET lots.

Conclusions

A corrective equation was developed for analyzing Sb content in extruded PET sheet which would not have otherwise been accurately quantitated as the sheet was far below the

infinite thickness required for accurate and repeatable measurements. Sb was selected due to its abundance in PET as a polymerization catalyst. The predictive model was able to accurately quantify Sb concentrations in 94% of samples analyzed. XRF is a rapid, non-destructive method, and can easily monitor materials in a continuous system. If applied to an online, post-extrusion system XRF could play a vital role in monitoring heavy metal content in extruded plastics thereby reducing the time and costs associated with traditional benchtop elemental analysis. This research identified an equation capable of adjusting the measured concentration of antimony in PET sheet by XRF, below the infinite thickness, to measured concentration via ICP-OES within a 20% margin of error. Similar correction equations for the analysis of the CONEG-regulated heavy metals lead, cadmium, chromium, and mercury are under current investigation.

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CHAPTER 4: GENERAL CONCLUSIONS

General discussion

The research in chapter 2 showed that significant differences were obtained between different microwave digestion protocols, using the same samples, for lead and antimony. Significant differences were not realized in cadmium and chromium due to large amounts of variation in concentrations of these heavy metals. It is thought that the significant differences observed between lead and antimony but not cadmium and chromium is correlated to sample concentration. Concentrations of heavy metals that are close to the limit of quantification show greater error, due to the uncertainty at those levels. Since lead and antimony were more abundant in polyethylene terephthalate materials used in this study, less error was observed from the analysis of those elements. It is recommended that a digestion protocol which yields digestates free from any particulates be used for heavy metals analysis. Furthermore, current regulations have set a limit of 100 ppm for heavy metals lead, cadmium, hexavalent chromium, and mercury. Though large amounts of error were observed from these analyses, the largest sum of heavy metals observed was 2.25 ppm, when accounting for variation. As such, there appear to be no immediate concerns associated with using incomplete digestions for total heavy metals analysis as it relates to regulatory levels.

Chapter 3 provided a method for the determination of antimony concentrations in thin (0.3 – 0.5 mm) extruded polyethylene terephthalate sheet using energy dispersive X-ray fluorescence (EDXRF). Samples that are too thin yield intensity outputs that do not represent the accurate sample concentration, when compared to a calibration curve. It was determined that observed concentration increases as sample thickness increases. In order to determine

more accurate representation of the true concentration, the ratio of the observed EDXRF concentration was correlated to the observed determined by inductively coupled plasma (ICP) concentration and used to develop a corrective equation. The equation obtained from the fitted model was used to evaluate nine unknown PET samples. From these nine samples, four repeated measures (36 total measurements) of each were analyzed by both EDXRF and ICP. Each output from EDXRF was used to predict the ICP output, prior to analysis. Of the 36 total measurements, 34 were accurately predicted by the model. This research has the potential to impact online monitoring of extruded plastic by making EDXRF a more accurate and reliable instrument for analysis of antimony in thin plastic sheet.

Recommendations for future research

The analysis of microwave digestion methods yielded mixed results in the current study. Significantly different results were obtained from the analysis of lead and antimony in PET, but cadmium and chromium were not significantly different. The reasons for this remain inconclusive. It was hypothesized that digestion methods which required filtering, such as that by Westerhoff *et al.* [1] would yield significantly lower concentrations than digestion protocols that did not require filtering. With mixed results in the current study, we were not able to definitively state whether our hypothesis was correct. In the current study, an inductively coupled plasma optical emission spectrometer (ICP-OES) was used for the analysis of lead, cadmium, chromium, and antimony. Since lead and antimony were well above the limit of quantification (LOQ), determining the concentrations of these elements was feasible. However, several samples tested for cadmium and chromium were below the LOQ for ICP-OES (1.53 and 11.56 µg/kg, respectively). As such, it is recommended that future analyses utilize an ICP mass spectrometer (ICP-MS) as used by Perring *et al.,* Carneado *et al*., Keresztes

et al., and Pereira *et al.* [2-5] as this would provide the quantification limits necessary to evaluate cadmium and chromium properly.

Energy dispersive X-ray fluorescence (EDXRF) provided a rapid method for the determination of antimony concentrations in thin PET sheet when a corrective equation was applied. The equation was developed by assessing the concentration of antimony in PET at varying thicknesses and fitting the data to a generalized least squares regression model. However, efforts to quantify the Coalition of Northeastern Governors (CONEG) – regulated heavy metals (lead, cadmium, hexavalent chromium, and mercury) was not studied. From what we learned about the content of these elements in PET in the first study, it is thought that concentrations of these elements may be too low for the sensitivity of EDXRF instrumentation. Turner and Solman had difficulty quantifying cadmium, chromium, and mercury in their analysis of marine litter using a portable XRF analyzer [6]. In several studies, EDXRF proved useful as a quality control measure for monitoring unexpected "spikes" in heavy metals on a processing line [7-9]. As such, it is recommended that dosed samples of varying concentrations, both above and below the limit of quantification of EDXRF, be used to develop a corrective equation for these elements. While normal concentrations would not be detectable, any spike in concentration that is above the limit of quantification should be easily detected using this methodology.

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APPENDIX A. SUMMATIONS OF THE CONEG HEAVY METALS

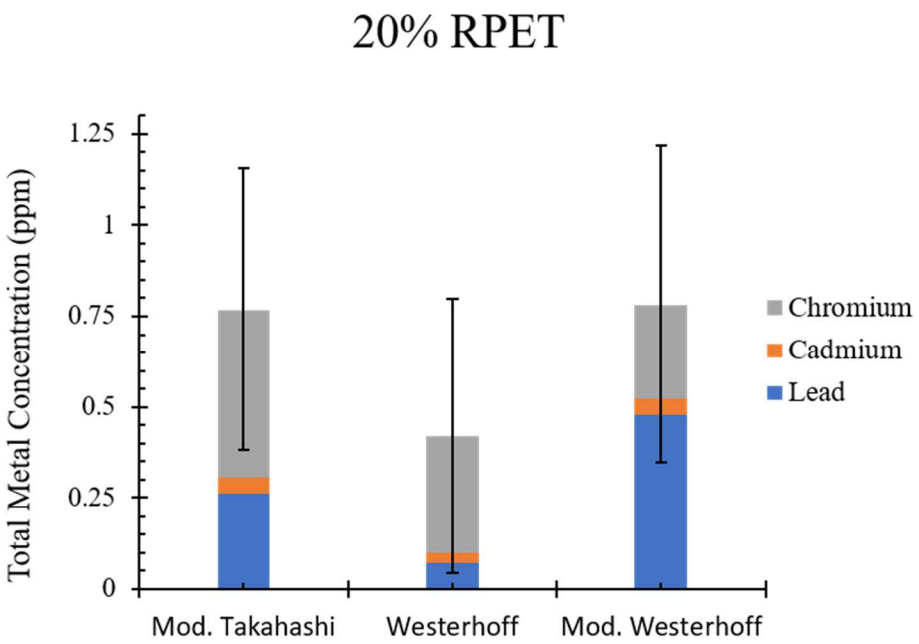

Figure A1. Sum of CONEG heavy metals for 20% RPET, digested using three methods. Error bars represent the sum of heavy metal standard deviations, for each method.

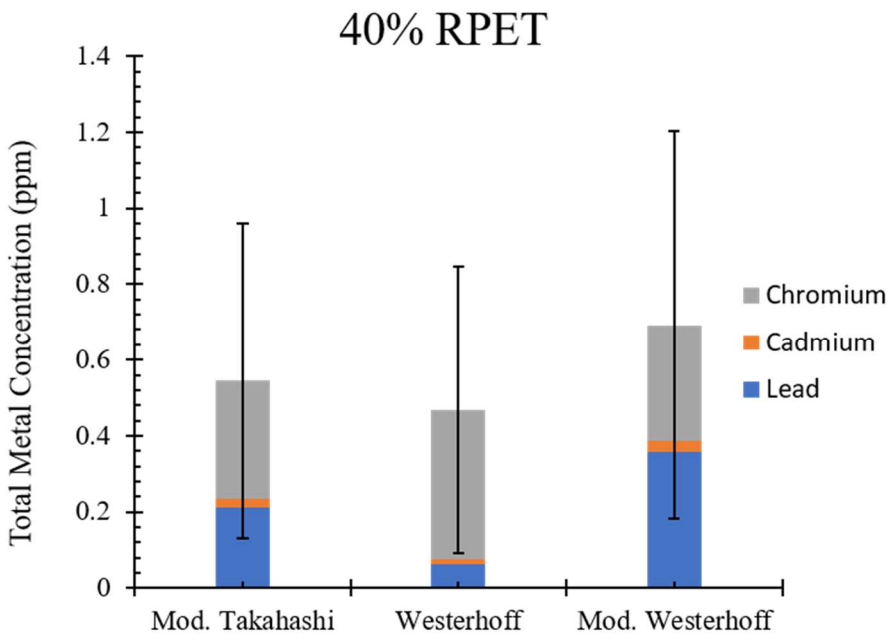

Figure A2. Sum of CONEG heavy metals for 40% RPET, digested using three methods. Error bars represent the sum of heavy metal standard deviations, for each method.

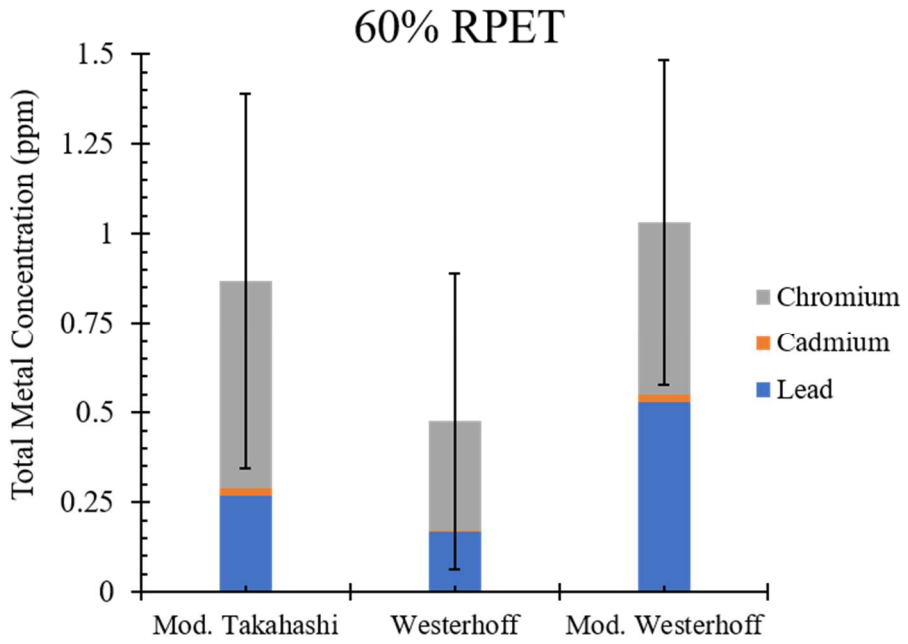

Figure A3. Sum of CONEG heavy metals for 60% RPET, digested using three methods. Error bars represent the sum of heavy metal standard deviations, for each method.

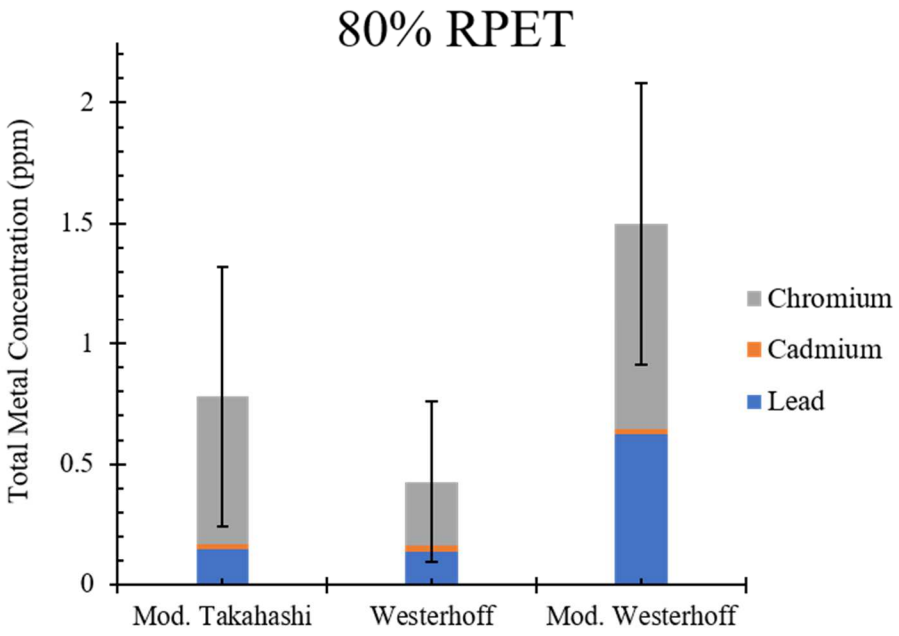

Figure A4. Sum of CONEG heavy metals for 80% RPET, digested using three methods. Error bars represent the sum of heavy metal standard deviations, for each method.

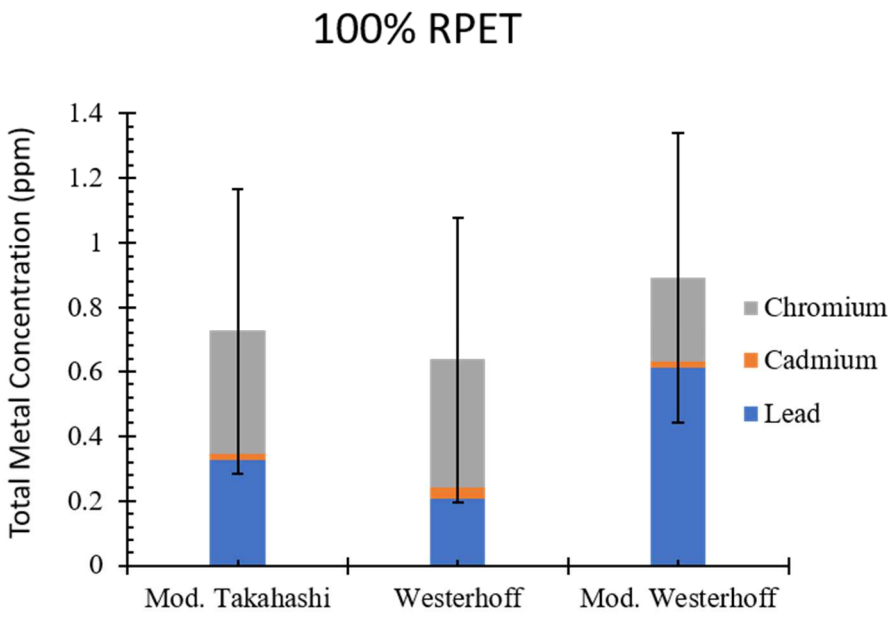

Figure A5. Sum of CONEG heavy metals for 100% RPET, digested using three methods. Error bars represent the sum of heavy metal standard deviations, for each method.

APPENDIX B. EDXRF PREDICTIVE DATA SUMMARY

Table B1. Raw data results for the predictive analysis of EDXRF data

Unknown Sample ID	Tech. Rep.	Thickness (mm)	Antimony by XRF (ppm)	Predicted ICP	Actual ICP	Margin of Error (-20%)	Margin of Error (+20%)	Within 20%?
A	1	0.470	6.9	163.0	201.8	161.4	242.1	YES
A	2	0.484	7.8	179.8	205.8	164.7	247.0	YES
A	3	0.475	8.0	186.9	201.1	160.9	241.4	YES
A	4	0.472	7.6	179.5	203.5	162.8	244.2	YES
B	1	0.346	7.1	227.6	256.6	205.2	307.9	YES
B	2	0.345	7.5	238.5	258.6	206.9	310.3	YES
B	3	0.353	7.6	238.2	265.4	212.3	318.4	YES
B	4	0.347	6.7	214.2	256.5	205.2	307.8	YES
C	1	0.494	9.6	217.1	215.1	172.0	258.1	YES
C	2	0.497	9.2	206.7	214.5	171.6	257.4	YES
C	3	0.519	10.8	231.3	212.6	170.1	255.2	YES
C	4	0.507	10.1	222.5	209.9	167.9	251.9	YES
D	1	0.545	12.2	250.4	209.6	167.7	251.6	YES
D	2	0.544	11.6	238.3	207.5	166.0	249.0	YES
D	3	0.547	11.4	233.1	208.0	166.4	249.6	YES
D	4	0.555	11.6	233.5	208.5	166.8	250.1	YES
E	1	0.334	6.7	222.4	225.4	180.4	270.5	YES
E	2	0.339	6.4	207.1	228.0	182.4	273.6	YES
E	3	0.332	7.0	231.0	228.2	182.5	273.8	YES
E	4	0.329	6.7	225.4	229.0	183.2	274.8	YES
F	1	0.534	6.1	127.1	163.6	130.9	196.3	NO
F	2	0.521	7.4	157.8	163.1	130.5	195.7	YES
F	3	0.524	6.7	142.2	167.0	133.6	200.4	YES
F	4	0.530	6.7	141.9	165.1	132.1	198.1	YES
G	1	0.512	9.6	209.5	227.4	182.0	272.9	YES
G	2	0.513	9.8	213.5	229.2	183.3	275.0	YES
G	3	0.521	10.3	219.7	224.8	179.9	269.8	YES
G	4	0.503	10.7	237.5	227.0	181.6	272.4	YES
H	1	0.533	9.7	202.4	207.5	166.0	249.0	YES
H	2	0.544	10.1	208.3	209.5	167.6	251.4	YES
H	3	0.536	10.9	228.2	210.9	168.7	253.1	YES
H	4	0.526	12.1	257.4	207.0	165.6	248.4	NO
I	1	0.526	9.3	198.0	192.5	154.0	231.1	YES
I	2	0.504	9.4	208.5	192.6	154.1	231.1	YES
I	3	0.508	9.2	201.7	194.0	155.2	232.8	YES
I	4	0.514	8.7	188.3	193.4	154.7	232.1	YES